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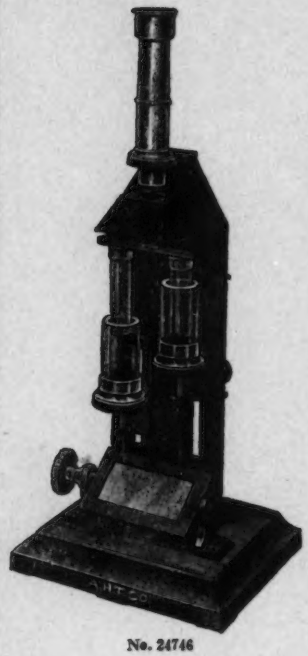

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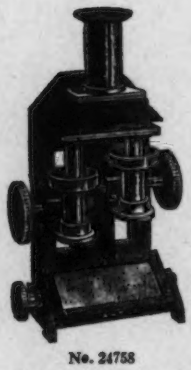

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THE AMERICAN
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THE EFFECT OF ETHER ANAESTHESIA ON THE ELECTRICAL ACTIVITY OF NERVE

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From the Laboratory of Physiology in the Harvard Medical School

Received for publication, March 27, 1916

INTRODUCTION

The experiments herein described were performed as a preliminary control with reference to an investigation now in progress in this laboratory on the effect of ether anaesthesia on the afferent impulses in the brain stem.[2] At Dr. Cannon's suggestion it was planned to determine by the recording of action currents with the string galvanometer, whether general surgical anaesthesia blocks the afferent impulses arising from peripheral stimulation at the synapses (or cell bodies) through which they must pass to reach the cerebrum and cerebellum. In view of the uncertainty which still exists as to the question whether the electrical disturbance is an inevitable accompaniment of the nerve impulse, it was deemed necessary, first, to determine whether by any treatment with ether it was possible to abolish the action current while still the nerve could be shown by other methods to be capable of conducting a nerve impulse. If this were the case, it is evident that abolition of action currents by ether anaesthesia would not prove abolition of nerve impulses.

We were led by an observation made by one of us in another series of experiments and reported with Gregg[3] to suspect that profound general anaesthesia might so affect a nerve trunk as to abolish the action

[1] We wish to thank Mr. M. Fremont-Smith for assistance in the first two experiments.

[2] A preliminary report on this investigation has already been made; see Forbes and Miller: This Journal, 1916, xl, 148 (proceedings).

[3] Forbes and Gregg: This Journal, 1915, xxxix, 194.

current. On this occasion a cat was subjected to extremely profound etherization during the operation of decerebration. Immediately after the completion of this procedure, the peroneal nerve was dissected out and connected with the galvanometer. On stimulating the nerve we failed to detect any action current. The nerve was then immersed for over an hour in mammalian Ringer solution at room temperature. It was then connected again with the galvanometer and this time yielded action currents on stimulation. This led us to suppose that the previous absence of electrical disturbance might in some way have resulted from the high concentration of ether in the blood, and that subsequent immersion in Ringer solution might have caused the elimination of ether and consequent restoration of a normal electrical condition. In view of our subsequent experimental results (to be here described) we are led to believe that the absence of action currents in this case was due to some accident in technique or experimental condition which was not noted, or to some other obscure cause not necessarily related to the use of ether.

In reviewing the literature we are concerned with facts bearing (1) on the question of the effects of ether upon nerve trunk activity in general and (2) on the question of the separability of action current and nerve impulse under any conditions. In regard to the first question, Cushny[4] states that nerves are not affected by ether when inhaled, but he cites Waller as having shown that when a frog's nerve is exposed to ether vapor in weak dilution, "its irritability is at first increased," while strong vapor temporarily abolishes the excitability. Borrutau[5] has investigated the effect of narcotics on the action current in frog's nerve as recorded with the capillary electrometer. He reports that alcohol, ether, chloroform and cocain produce no pronounced lengthening of the duration of electrical negativity; though in the course of their depressing effect there is retardation of conduction and delay in the subsidence of the action current. His electrometer records illustrate the effect of ether narcosis, a reduction of the action current occurring after five minutes and complete abolition after ten minutes; subsequent restoration occurring fifteen minutes after the withdrawal of ether.

In regard to the second question, the separability of action current and nerve impulse, Gotch[6] has argued that the nerve impulse may in

[4] Cushny: Textbook of Pharm. and Therap., 1906, p. 163.
[5] Borruttau: Pflüger's Arch., 1901, lxxxiv, 350.
[6] Gotch: Journ. Physiol., 1902, xxviii, 51, etc.

certain cases occur without electrical concomitant. Other investigators have discussed the same question, including Wedensky[7] and Borruttau[8] who have contended that the electrical disturbance is inseparable from the nerve impulse. The arguments on both sides have been reviewed by Lucas[9] and the conclusions more recently summarized by Forbes and Gregg.[10] It will suffice to state here that all efforts to prove that either the nerve impulse or the electrical disturbance which normally accompanies it, can occur without the other, have failed. The preponderance of evidence so far accumulated supports the view that the electrical disturbance is an inevitable adjunct of functional activity.

One more fact in regard to the estimation of functional activity in the nerve trunk must be noted before proceeding to the description of our experiments. Meltzer and Auer[11] found that ether inhalation in the dog reduces the height of contraction of skeletal muscle in response to stimuli applied directly or to the motor nerve. The muscle no longer responds with tetanic contraction to rapidly repeated stimulation of the nerve, and shows evidence of increased fatigue. The authors infer that ether exerts an action on the motor endings of the nerve similar to that of curare. This result obviously furnishes no evidence as to the effect of the drug on the nerve trunk, inasmuch as nerve fibre and nerve ending are physiologically distinct, but it indicates a factor which must be reckoned with in interpreting observations on the functional activity of the nerve trunk. Muscular contraction following stimulation of the nerve is proof of the occurrence of the nerve impulse, but absence of contraction is no proof of functional inactivity in the nerve.

METHOD

Two methods were adopted in our experiments. One was to determine whether the most profound anaesthesia which could be induced in the cat by inhalation of ether sufficed to abolish or clearly reduce the action current in a nerve trunk directly stimulated; and if it did abolish the action current, whether it simultaneously abolished the contraction in the innervated muscle, this being the only index of functional activity in the nerve. The second procedure, substantially that of Weden-

[7] Wedensky: Pflüger's Arch., 1900, lxxxii, 132.
[8] Borruttau: Loc. cit., 325.
[9] Lucas: Proc. Roy. Soc., 1912, lxxxv, 502-508.
[10] Forbes and Gregg: Loc. cit., 215-217.
[11] Meltzer and Auer: Journ. of Pharm., 1914, v. 521.

sky,[12] was to expose the nerve trunk of either cat or frog to the direct action of ether vapor and note whether at any time contraction in the innervated muscle persisted when the action current of the nerve was no longer obtainable. Since the muscular contraction was to be used as an index of functional activity in the nerve, it was necessary to record the action currents diphasically; and in order to separate the phases far enough to admit of well defined galvanometric excursions, the leading-off lectrodes were placed as far apart on the nerve as they conveniently could be without bringing the proximal lead near enough the stimulating electrodes to produce confusing "artefacts."[13]

The apparatus, consisting of a Cambridge string galvanometer and photographic recording camera, has been described in detail in a previous paper.[14] In these experiments, as in the last few reported in that paper, an arc lamp was used for illumination, and the large cylindrical lens employed with the Nernst lamp was thus dispensed with. The galvanometer was provided with the low resistance magnet coil excited by eight Edison cells. The platinum string, designated "String C" in the previous paper,[15] was used throughout. The wiring and the stimulating apparatus were exactly as described therein.

When the effect of general anaesthesia on the nerve trunk in the cat was to be studied two procedures were employed. In the first of these the sciatic nerve of the anaesthetized cat was laid bare from hip to knee, but was not at first dissected out from the surrounding tissues, the chief aim being to avoid disturbing its blood supply. For a distance of 2 or 3 cm. at the hip that portion of the sciatic nerve which branches off farther down as the peroneal, and which can be plainly seen as a distinct bundle, was carefully dissected away from the rest of the nerve, ligated and cut at the most central point so dissected. The blood supply to the major part of the nerve between the hip and the knee is not disturbed by this operation.

A pair of non-polarizable boot electrodes was set up in the moist receiving chamber, previously described. A pair of platinum stimulating electrodes was fixed at the opposite end of the chamber from the opening through which the nerve was to be drawn in.

Ether was administered with a bottle through a tracheal cannula; the depth of anaesthesia was gauged by frequent observations of the

[12] Wedensky: Loc. cit., p. 139.

[13] See Forbes and Gregg: Loc. cit., 186, etc.; also fig. 18.

[14] Forbes and Gregg: This Journal, 1915, xxxvii, 121–132.

[15] Loc. cit., 122.

corneal reflex, the pinna reflex (retraction of the pinna evoked by pinching it with forceps) and the character of respiration. From time to time the separated nerve bundle at the hip was stimulated with a break shock and the resulting contraction of the tibialis anticus muscle noted. With the apparatus in readiness the ether was crowded on until respiration almost or wholly ceased, then the peroneal nerve was quickly dissected from the rest of the sciatic nerve and from the surrounding tissues all the way from the hip to its entrance into the tibialis anticus muscle. This dissection could readily be completed during the time that the animal was too deeply anaesthetized to breathe spontaneously,

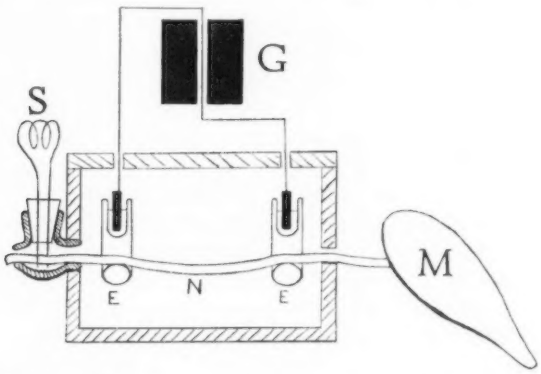

Fig. 1. Arrangement of cat's peroneal nerve in moist chamber for recording action currents. *N*, nerve; *M*, muscle; *S*, stimulating inductorium; *E.E*, "boot" electrodes; *G*, string galvanometer. The details of the galvanometer circuit, omitted here for simplicity, were exactly as shown in figure 1 of the paper cited, this Journal, 1915, xxxvii, 124.

and yet the animal could be revived by artificial respiration and thus maintained for further experimentation. As soon as the peroneal nerve was dissected out it was quickly laid across the non-polarizable electrodes in the moist chamber, and the ligatured central end was connected with the stimulating electrodes. Thus the nerve, having been separated from its blood supply when this was most heavily charged with ether, was arranged as indicated in figure 1, with leading-off electrodes between the point of stimulation and the muscle. Break shocks were then applied and the muscular contractions noted while the string galvanometer was used to detect the presence of diphasic action currents arising from the passage of the impulse over the leads in the moist chamber.

After this experiment was performed, it was, of course, impossible to use this nerve for a repetition of the same experiment, but it was possible to subject the nerve trunk to the direct action of the ether vapor. But in the two cases in which this was tried the results were unsatisfactory since failure in conduction appeared in the nerves, apparently unrelated to the effects of ether. For this reason our conclusions in regard to the direct application of ether vapor to nerve are based on our experiments with the frog's nerve-muscle preparation which will be described presently.

In the second procedure for the study of general anaesthesia the sciatic nerve in the etherized cat was exposed from the hip to the knee, and the whole nerve cut at the hip as far up as possible. The distal cut end was then ligatured to facilitate manipulation, and dissected from the surrounding tissues far enough to admit of the application of a pair of Sherrington shielded electrodes. The peroneal branch was carefully freed from the surrounding tissues for a sufficient distance between its departure from the popliteal branch and its entrance to the tibialis anticus muscle to permit the application of a boot electrode without contact with other tissues. The tip of one boot electrode was then brought in contact with the sciatic nerve in the thigh region, and the other was applied to the peroneal nerve at the knee. The galvanometer was thus connected with the nerve in a region where the blood supply was undisturbed. Etherization was then crowded to the point of abolishing spontaneous respiration, and frequent records were taken of the action currents resulting from single shocks as the anaesthesia deepened. In one experiment with this method the nerve to the hamstring muscles, branching from the sciatic between the stimulating electrodes and the proximal lead, was not cut, and because the uncut nerves with this arrangement provided an adequate conducting path, the action current of the hamstring muscles entered confusingly into the records. In a subsequent experiment the hamstring nerve was cut and the disturbing factor thus eliminated. It should be noted that with this mode of application of electrodes a shift of contact results from the contraction of the muscles, thus causing an excursion of the string due to change in demarcation current. But this is of no consequence when the action current is recorded on a rapidly moving film, for the latencies of nerve action current and mechanical disturbance are so different as to render it easy to distinguish between them.

In a final experiment the sciatic nerve was exposed but not dissected at all nor disturbed as to its circulation until the animal had

been etherized to the point at which respiration had ceased and the heart had almost stopped beating. The sciatic nerve was then quickly dissected out, severed at hip and knee, and placed at once on stimulating and leading-off electrodes.

When the second general method was employed, viz., that of exposing a nerve trunk to ether vapor, the frog's nerve-muscle preparation (sciatic-gastrocnemius) was placed in a moist chamber with a pair of boot-electrodes for leads between the stimulating electrodes and the muscle. After one or two diphasic action currents had been recorded, cotton soaked in ether was placed on the floor of the moist chamber under the nerve and between the stimulating electrodes and the proximal lead. It was placed here to insure greater concentration of ether vapor in the neighborhood of the proximal lead than at the neuromuscular junction; for if the action of the ether were to occur at the junction, the muscle might cease to contract before that part of the nerve trunk from which the action current was led had been affected. Records were taken at frequent intervals as the ether acted, and the muscle was watched closely, till contractions ceased. One or two more records were taken after the cessation of contraction, and then the ether was removed and the nerve permitted to recover. The experiment was then repeated. Each preparation studied was etherized in this way twice.

EXPERIMENTAL RESULTS

The results will be described in the same order as the procedures. First, in regard to gauging anaesthesia by reflexes, it was found that the corneal reflex disappeared with a moderate degree of anaesthesia, while the pinna reflex persisted much longer, lasting in almost every case about as long as respiration. There was a somewhat notable uniformity in this approximately simultaneous disappearance of pinna reflex and spontaneous respiration. In all experiments with general anaesthesia it was found that muscular contraction in response to motor nerve stimulation remained vigorous at all depths of anaesthesia, even after respiration had ceased altogether. Therefore, it is clear that the nerve impulse still passes in the motor fibres under these conditions. The next question is whether the action current persists with it. A fairly conclusive answer was found in these experiments in which the peroneal nerve was dissected from its blood supply during profound etherization.

In one of these the nerve was isolated, as etherization was being

pushed, some time after the corneal reflex had disappeared, but while the pinna reflex was present and respiration only slightly impaired. When the nerve was connected with the galvanometer and stimulated vigorous diphasic action currents were recorded. Thus it is evident that at the somewhat profound surgical anaesthesia here tested the electrical disturbance is not abolished.

In another experiment with this method the peroneal nerve was not dissected from its blood supply till respiration had ceased; the dissection was then performed while artificial respiration was being applied to revive the animal. Figure 2 shows the action currents obtained one minute (*A*) and five and a half minutes (*B*) after the dissection was completed, the dissection taking three and one-half minutes from the cessation of respiration. These action currents are approximately as large as would ordinarily be obtained from the peroneal nerve of a decerebrate cat that has fully recovered from the anaesthetic. It is clear, then, that a nerve cut off from its blood supply at a time when this contains ether in sufficient quantity to abolish respiration, not only performs its physiological function but exhibits a substantially normal action current as well.

Confirmatory evidence was sought by the method described next in order, viz., the recording of the action current from the sciatic nerve whose blood supply remained intact while etherization was pushed to the limit. In the first experiment, that in which the hamstring nerve was not cut, the results were rendered confusing, as already noted, by the entrance of the action current of the hamstring muscles.

In the experiment in which the hamstring muscles were thrown out of action by section of their motor nerve we obtained a most satisfactory confirmation of the conclusion already arrived at by the other method. With electrodes applied as described, one to the sciatic nerve about 2 cm. below the hip, one to the peroneal nerve at the knee, action currents were obtained at all depths of etherization. Figure 3 shows one (*A*) obtained under moderate anaesthesia while respiration was normal, and another (*B*) four minutes later after respiration had ceased.

In the final experiment on general anaesthesia in which a cat was etherized to death, and the sciatic nerve dissected almost at the moment when circulation ceased, the most convincing evidence was obtained. When the nerve was removed from the animal's body and laid across stimulating and leading-off electrodes with a crushed point between the latter, action currents were recorded which compare well with those of the sciatic nerve removed from the decerebrate animal without anaesthesia. The record of one of them is shown in figure 4.

The results of the experiments in which ether vapor was applied directly to the exposed frog's nerve were also uniformly clear in their bearing on the question at hand. As the contractions grew weaker the action currents grew smaller till finally both were abolished, but in every instance contraction disappeared first. In one instance records were taken showing the persistence of the action current after contractions had ceased, and, when the ether had been withdrawn from the chamber, the presence of a well defined action current a few seconds

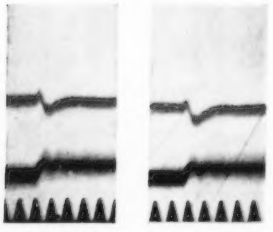

Fig. 2

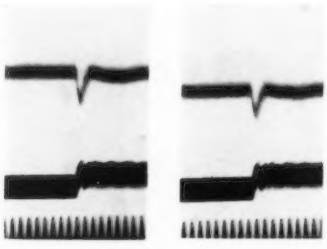

Fig. 3

Fig. 4

Fig. 2. Experiment 5. Diphasic action currents in cat's peroneal nerve; see text. In this and all other photographic records the top line shows the excursions of the string. The second line shows the time of stimulation; a rise in this line shows the break of the primary current. The small oscillations following the break are vibratory and do not indicate secondary closure of the circuit. The bottom line records time; each complete vibration = 0.01 second. In all except figure 3 upward excursion of the string means fall of potential in proximal lead.

Fig. 3. Experiment 7. Action currents in cat's sciatic nerve; see text. In this experiment the lead wires to the galvanometer were reversed, thus causing the string to move down instead of up.

Fig. 4. Experiment 8. Monophasic action current of cat's sciatic nerve; see text.

after contraction was first seen (fig. 5 *A*). In other cases the action current remained appreciable, though very small, in several successive records taken after contraction had ceased (fig. 5, *B*). In no instance was contraction seen when the action current was absent.

The fact that action currents are to be found in the nerve after contraction had ceased does not, of course, prove any lack of parallelism between the electrical disturbance and functional activity in the nerve.

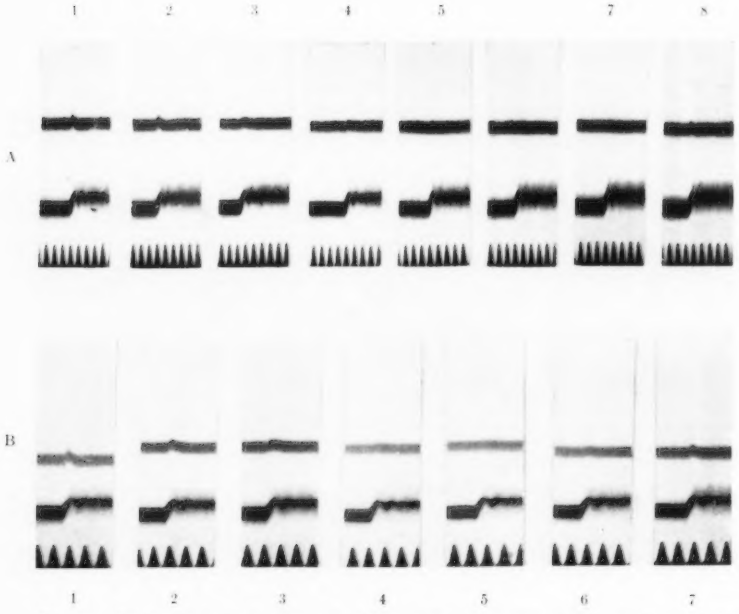

Fig. 5. Diphasic action currents of frog's sciatic nerve showing effect of ether vapor.

A. Experiment 4. *1*, before ether was introduced, contraction normal; *2*, two minutes after ether was introduced, contraction fair; *3*, three minutes of ether, contraction weaker; *4*, $3\frac{3}{4}$ minutes ether, contraction very weak; *5*, $4\frac{1}{4}$ minutes ether, no contraction; *6*, five minutes ether, no contraction. *7*, $\frac{1}{2}$ minute after ether was removed from chamber, no contraction. *8*, $1\frac{1}{2}$ minute after removal of ether, slight contraction.

B. Experiment 3. *1*, one minute of ether, normal contraction; *2*, $2\frac{1}{2}$ minutes of ether, contraction weaker; *3*, three minutes ether, contraction very weak; *4*, $3\frac{1}{4}$ minutes ether, no contraction; *5*, four minutes ether, no contraction; *6*, $1\frac{1}{4}$ minute after removal of ether, contraction small; *7*, two minutes after removal of ether, contraction small.

Even if, as was intended, the action of ether was more intense near the stimulating electrodes than at the nerve ending, it is quite possible that the decrement imposed on the nerve impulse by the drug was such that it failed to pass the neuromuscular junction and excite the muscle, although persisting throughout the region where the galvanometer leads were applied.[16] On the other hand, the failure in any instance to obtain muscular contraction without a simultaneous action current in the nerve, although the ether was regularly introduced proximal to the leads, supports, so far as it goes, the view that the nerve impulse cannot occur without its concomitant electrical disturbance. At all events, it supports the findings in the case of general anaesthesia, and leads to the conclusion that it is impossible by any application of ether to abolish the electrical disturbance in nerve while the tissue remains functionally active. The action current is probably a valid criterion of function.

SUMMARY

1. As a preliminary to an investigation of the effect of ether anaesthesia on afferent impulses going to the brain, a study has been made of its effect upon the action current in a nerve trunk subjected to direct stimulation.

2. Two methods have been employed. One was to etherize a cat and see whether at any depth of general anaesthesia the action current in a motor nerve directly stimulated, could be abolished. The second was to apply ether vapor directly to an exposed frog's nerve and see whether at any time the action current could be abolished while the nerve was still shown to be functionally active by contraction in the innervated muscle.

3. We find that even when etherization in the cat is pushed to the point of abolishing respiration and causing death, the nerve trunk remains functionally active and exhibits what appear to be essentially normal action currents.

4. With direct application of ether vapor to the isolated nerve-muscle preparation, the action current in nerve regularly persists at least as long as contraction in the muscle, and in almost every instance longer.

5. The evidence, so far as it goes, supports the view that nerve impulse and electrical disturbance are inseparable. It leads us to believe that, at any rate, the action current is a safe criterion of the presence or absence of the nerve impulse in connection with the administration of ether.

[16] Adrian: Journ. Physiol., 1912, xlv, 389; 1913, xlvi, 385.

A METHOD FOR MAINTAINING AN ARTIFICIAL CIRCULATION THROUGH THE TIBIA OF THE DOG, WITH A DEMONSTRATION OF THE VASOMOTOR CONTROL OF THE MARROW VESSELS

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From the Physiological Laboratory, Johns Hopkins University

Received for publication, March 30, 1916

INTRODUCTION

The inaccessible nature of the bone marrow seems to have preserved it from attempts at direct physiological study. With the exception of work by Franz Müller(1) we have found no efforts towards such an end. This observer in 1901 examined blood taken directly from the nutrient vein of the tibia, and found that if his operation was performed with no interruption of arterial flow and with no hemorrhage, this blood corresponded entirely with blood taken from other parts of the same animal. But if he clamped the nutrient artery from twenty to thirty minutes, the specimens then contained many normoblasts. The same result, though less marked, could be secured by making the animal breathe an oxygen poor atmosphere. The article aims to clear up certain phases of the problem of oxygen lack and red cell formation. It makes no mention of the other principal marrow product, the leucocyte. The full significance of these results will be discussed at a later date, since they seem to have a very direct bearing on the mechanical factors involved in the appearance of new cells in the circulation.

Müller also mentions the fact that nerves have been found passing to the marrow vessels. That the limbs possess a poor vasomotor mechanism as compared with the abdominal vascular area is common knowledge, but the possibility of a complete and very effective supply to the bone marrow has entirely escaped physiological observation. Gros (2), in 1845, first described the existence of such nerves. Variot and Remy (3), in 1880, attempted to amplify his observations. But the methods then at hand were not adequate for the task. Ottolenghi (4), in 1901, with the advantage of the methods of Golgi and Ehrlich made

a very thorough study of the nerves to the marrow. He examined material from man, sheep, dog, guinea pig, rabbit and chicken, and concludes:—

1. The marrow is richly supplied with medullated and non-medullated fibres.

2. These nerves form fine plexuses in the walls of the blood vessels, many ramifications reaching the capillaries.

3. In the marrow pulp there are many medullated and non-medullated fibres passing eventually to distant vessels.

4. The existence of special nerve terminations about independent marrow elements cannot be settled.

Myelinated fibres in this region can only be afferent and suggest the possibility of specialized reflexes from the marrow.

METHOD OF PERFUSION

After many dissections the tibia of the dog was found to be the bone best suited for perfusion. Similar experiments can be done upon the femur but the operation is more difficult and the bulk of tissue perfused not so large. Dogs weighing about 8 kgm. are desirable but, if necessary, smaller animals may be used when the operator is thoroughly familiar with the anatomy of the part. A skin incision from a point 1 inch above the knee joint to a point 2 inches above the ankle joint, along the line of the inner border of the tibia, exposes the fascia above the bone and permits the operator to carry out his dissection along the inner margin. Care should be taken not to cut or tie the saphenous vein as it receives radicles from the lower extremity. The popliteus muscle is cut away from its insertion, and the popliteal artery and vein exposed as they pass down between the condyles of the femur. At all times the operator must keep close to the bone, cleaning off all the muscle attached to the periosteum, but not removing this membrane. The thick fascia covering the flexor communis digitorum is cut close to the bone and the origin of this muscle is carefully cleared away by blunt dissection. The nutrient artery usually leaves the popliteal artery as one branch of a short small trunk appearing about three-fourths of an inch below the knee joint, and runs obliquely downwards, passing under the tibial periosteum near the external border about one-third of the way down the shaft. It traverses the belly of the flexor communis digitorum, and when the origin of this muscle is removed the artery with the vein beside it will be seen. Occasionally

the artery comes off independently somewhat lower down. The nerve to the marrow, a branch of the tibial, is extremely small and lies in close relation to the nutrient artery. Figure 1 indicates the relations which obtain after the dissection is completed and all the muscular branches in the neighborhood ligated. Having made such exposure, a cannula is placed in the popliteal artery below the egress of the nutrient artery, the perfusion started, and the fluid allowed to flow up the popliteal artery against the normal blood stream. A ligature which has been placed around the popliteal above the nutrient artery is now tied, and the perfusing fluid is shunted through the latter artery without at any time interrupting the flow of fluid to the bone. In order to eliminate the possibility that the reactions hereafter described are due to contractions of the nutrient artery outside the bone, a cannula of the type shown in figure 2 has also been used. With the perfusing fluid running, such a cannula is passed into the popliteal below the nutrient artery and its point gently guided up into the mouth of the nutrient artery, down which it is cautiously pushed until the point reaches the periosteum. There are no differences in the type of result obtained by these two preparations.

If such procedures are properly carried out is the marrow completely perfused?

The tibia in common with other long bones has three sources of blood supply:

1. By minute periosteal arteries from fascial and muscular twigs which pass near the bone.

2. By a number of moderate sized vessels which enter the extremities of the bone through small foramina usually in the line of the attachment of the joint capsules.

3. By the nutrient artery. This is unquestionally the main source.

At the present time there is no basis upon which to found a belief that perfusion through the nutrient artery will keep all the marrow alive, but we can ascertain that all the marrow, except at times the extreme upper posterior part of the tibial head, is thoroughly bathed by the perfusing fluid. The lower extremity receives practically no blood save by the nutrient artery. There are usually two and sometimes three very short small branches which pass directly from the popliteal artery to the upper posterior part of the head. These are hard to see in the living preparation as any disturbance of the popliteal artery breaks them. It is possible to take advantage of their presence by passing the ligature, which shunts the perfusing fluid into the bone,

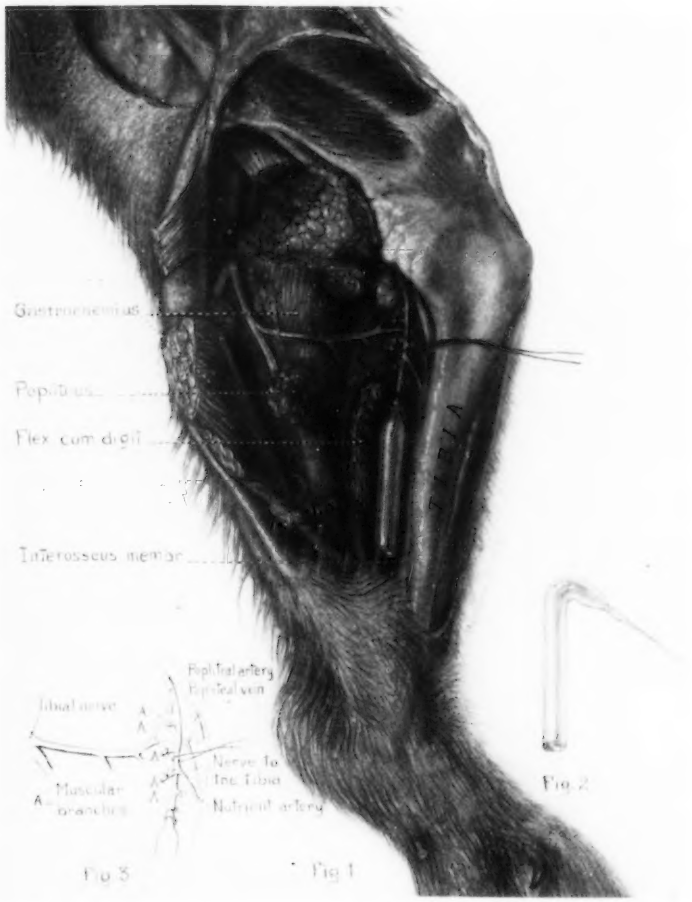

Fig. 1. Dissection of the nutrient artery and of the nerve to the marrow. The tibial nerve is displayed fully in order to show the origin of the nerve to the marrow.

Fig. 2. Special cannula for insertion into the nutrient artery.

Fig. 3. Key to figure 1.

above the head of the tibia, but in the experiments here detailed this was not done.

The long bones possess three sets of veins corresponding to the arteries, and though the nutrient vein carries the main effluent, leakage from vessels throughout the periosteum is a constant difficulty. A preparation can be made in which the venous return can be kept intravascular throughout and can be collected from the vein, but this has been unnecessary in these experiments. In this work when the perfusion has been started the common iliac artery is tied, the tibia is disarticulated at the knee and ankle and is cut away from its muscular attachments. The isolated bone, to which a circulation has thus been uninterruptedly supplied, is then placed in a shallow trough with an outlet tube at one end. Now all inflow reaches the marrow through the nutrient artery and all outflow falls in the trough and drops from the outlet tube. We have made use of a simple form of constant pressure and continuous flow perfusion apparatus and have employed oxygenated Ringer's solution as the perfusing fluid. With such an apparatus variations in the outflow under constant inflow pressure can mean only variations in the calibre of the vessels perfused.

EXPERIMENTS

No. 19. Action of epinephrin. Dog, weight 14.2 kgm. Anesthesia; Luminal-Sodium* administered intraperitoneally. Perfusion with oxygenated Ringer's solution started at 3.51 p.m. Bone removed at once. Outflow recorded in drops.

Time	*Drops of outflow in 30 seconds*		*Time*	*Drops of outflow in 30 seconds*	
4.34	64	Pressure 115 mm. Hg.	4.46	6	
			4.48	11	
4.35	64		4.49	16	
4.37	64		4.52	47	
4.38	63		4.55	51	
4.39		Epinephrin 0.000,02 gm.†	4.57	52	
			4.58	53	
4.39^{30}	11		5.00	52	
4.40^{30}	4		5.01		Epinephrin 0.000,002 gm.
4.42	2				
4.43	2		5.01^{30}	28	
4.44	1		5.02	4	

* Luminal-Sodium(5) gave entire satisfaction when given intraperitoneally dissolved in 0.9 per cent NaCl in the dose of 0.150 gm. per kgm.

† This and subsequent doses of epinephrin dissolved in Ringer's solution were injected through the rubber tubing leading to the inflow cannula. The dilution in which the drug reached the marrow vessels cannot be given.

Time	Drops of outflow in 30 seconds		Time	Drops of outflow in 30 seconds	
5.03	2		5.49	41	
5.06	0		5.51		Epinephrin
5.20	5				0.000,000,2 gm.
5.30	44		5.51^{30}	36	
5.44	40	Pressure 102 mm.	5.52	27	
		Hg.	5.53	28	
5.45	41		5.54	31	
5.48	42		5.58	44	

No dilatation with epinephrin could be observed under the conditions of this experiment. It must be recognized that the marrow vessels at the outset of such an experiment are in a condition of wide paralytic dilatation due to the severance of their nerve supply. This is probably exaggerated by constant pressure perfusion and by the poor oxygen carrying power of the Ringer's solution. A slight but rather doubtful degree of active dilatation was secured in another experiment in which ergotoxin phosphate was given to a dog until small doses of epinephrin gave a dilator reaction. At this point perfusion was started, the bone removed and epinephrin injected. The dilatation which resulted could be repeated by only one subsequent injection and could not be shown by any type of direct nerve stimulation. We have therefore no reliable evidence of an active dilator mechanism.

No. 25. Electrical stimulation of the nerve to the marrow. Dog, weight 13.6 kgm. Anesthesia; Luminal-Sodium administered intraperitoneally. Perfusion with oxygenated Ringer's solution.

Time	Cc. of outflow in 30 seconds		Time	Cc. of outflow in 30 seconds	
12.00	11.5	Pressure 125 mm.	12.05		Stimulation begun
		Hg.	12.05^{30}	9.8	
12.01	11.0		12.06	3.2	Stimulation ended
12.02	11.0	Electrode in posi-	12.07^{30}	5.0	
		tion	12.08^{30}	10.0	
12.04	12.0		12.11	10.0	

In this case a very large flow was secured and the experiment is given to indicate the extent to which the flow can be reduced by faradic stimulation of the nerve.

No. 27. Electrical stimulation of the nerve to the marrow. Dog, weight 20.2 kgm. Anesthesia; Luminal-Sodium administered intraperitoneally. Bone removed in usual manner. Outflow record with drop recorder. Nerve stimulated with faradic current between marks *A* and *B*. Figure 4 gives the record obtained in this experiment and presents the ordinary characteristics of vasomotor reactions, i.e., slow onset and slow disappearance.

It has been impossible to secure vasodilatation by slow weak stimulation. The mechanism is so sensitive that handling of the nerve, unless accomplished with the utmost precaution, causes constriction. The constrictor effect of epinephrin has been observed in eight different experiments and constriction on direct stimulation of the nerve has been observed in five. From these experiments we have given the above characteristic examples, and feel that they present ample verification of the existence of the large vasomotor supply which the anatomical investigations of Ottolenghi have indicated.

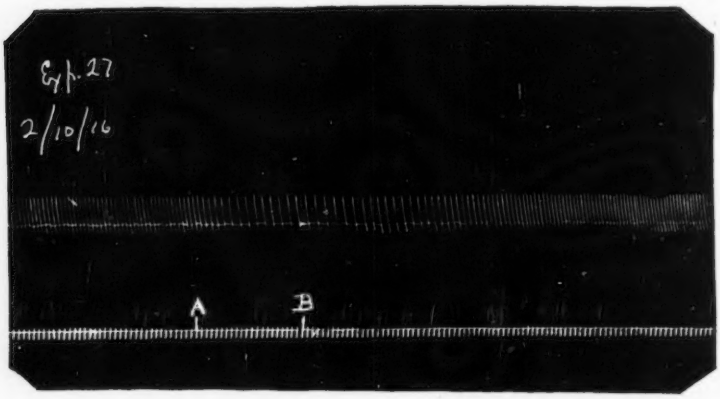

Fig. 4. Vasoconstriction on faradic stimulation of the nerve to the marrow. Stimulus applied between *A* and *B*. Upper line: drop recorder. Lower line: chronograph recording two second intervals.

Our present studies indicate that this intense nervous control has a marked influence on the cellular output of the marrow and experiments dealing with this aspect of the work are now in progress.

CONCLUSIONS

a. A method of perfusing the bone marrow is described.

b. The existence is demonstrated of vasomotor nerves to the marrow. These nerves respond on electrical stimulation and on injection of epinephrin by causing vasoconstriction.

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BLOOD PRESSURE IN HAEMORRHAGE AND ITS RESTORATION

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Haemorrhage lowers blood pressure, but the fall in pressure is not invariably proportionate to the amount of blood lost. As is stated by Pilcher and Sollmann, the relation of the fall of blood pressure to the amount of blood lost varies in each animal, but the median type is approached more or less closely in each case. As is further stated by the same authors, the low blood pressure level, or the blood pressure in shock, depends chiefly on the amount of blood lost and not to an important degree on the rapidity of the haemorrhage (1).

In performing the experiments that will be referred to in this paper we were actuated by a desire to learn just what results could be hoped for in endeavoring to restore the blood pressure to normal after haemorrhage. For experimental purposes rabbits were used. They were anaesthetized by the administration through the stomach tube of 1.5 cc. of paraldehyde per kilogram of body weight. The haemorrhage was accomplished by withdrawing from the femoral artery blood in the proportion of 5 cc. per kilogram of body weight, and continuing to withdraw equal amounts until the blood pressure was reduced to the desired level. Intravenous injections of normal saline solution were then made in amounts sometimes less than, in others equal to, and in still others greater than, the amount of blood lost.

In general it may be stated that removal from the circulation of 5 cc. of blood per kilogram is without influence on the blood pressure. Upon the withdrawal of the second portion of 5 cc. per kilogram blood pressure begins to fall and there is a fairly constant fall of pressure with the removal of each successive portion until 20 cc. or 25 cc. per kilogram have been withdrawn. The fall of blood pressure with the loss of each 5 cc. of blood per kilogram averages 6 mm. of mercury (Group 1). After 20 cc. or 25 cc. per kilogram have been removed the loss of more blood causes a more rapid fall in pressure. At this

point we found that each 5 cc. of blood lost per kilogram caused an average fall in blood pressure of 10 mm. (Group 2), and when 35 cc. to 40 cc. per kilogram had been lost the animal was in a condition of shock with a blood pressure varying in different animals from 22 mm. to 35 mm. of mercury.

If normal saline solution be injected during the first stage of haemorrhage, that is while the blood pressure is falling slowly, there is a rapid and permanent return to normal. This may be seen by reference to the tabulated report of group 1.

Group I

EXPERIMENT	HAEMORRHAGE IN CUBIC CENTIMETERS PER KILOGRAM	5	10	15	20	25	NORMAL SALINE IN CUBIC CENTIMETERS PER KILOGRAM	BLOOD PRESSURE AFTER INJECTION
A1	Blood pressure, 80	80	77	72	66	60	30	75
A2	Blood pressure, 84	82	76	69	61	53	25	73
A3	Blood pressure, 81	80	73	64	55		15	76
A4	Blood pressure, 84	80	71	66	59		20	82
A5	Blood pressure, 82	81	75	69			10	79

The first blood pressure recorded is that at the beginning of the experiment, before any blood had been withdrawn. The figures given for blood pressure represent millimetres of mercury.

If the injection of normal saline solution be made during the second stage of haemorrhage, that is during the period of rapid fall in blood pressure, the permanent return of pressure to normal can be accomplished, but the response is much slower than in the first stage. Immediately following the saline infusion the pressure rises 20 mm. or even 30 mm. In one case it rose 38 mm. After the initial rise the return to normal is slow and requires from twenty-four hours to forty-eight hours.

Group II

EXPERIMENT	HAEMORRHAGE IN CUBIC CENTIMETERS PER KILOGRAM	5	10	15	20	25	30	35	SALINE IN CUBIC CENTIMETERS PER KILOGRAM	BLOOD PRESSURE
B1	Blood pressure, 81	80	73	65	58	50	39	28	45	58
B2	Blood pressure, 85	82	74	66	58	53	40	30	35	55
B3	Blood pressure, 80	80	73	67	60	52	46	35	30	73
B4	Blood pressure, 86	84	80	71	63	51	41		30	61
B5	Blood pressure, 82	79	68	59	47	39	30		40	48

If the injection of saline solution be deferred until the third stage of haemorrhage, i.e., until collapse has occurred, the possibility of bringing about a permanent restoration of blood pressure is remote. The first effect of the saline injection is a rise of pressure, this rise usually being about 10 mm., though in one case that will be referred to again it was 20 mm. Repeated injections of large amounts of saline solution were without further effect under these conditions except in the case mentioned. In this case the method followed was the same as in four others. Blood pressure was reduced to 45 mm. of mercury by successive bleedings, the total amount of blood lost being 40 cc. per kilogram. Prompt administration was made of 50 cc. of saline solution per kilogram with rise in blood pressure of 20 mm. As the blood pressure began to decline a further injection of 50 cc. of saline solution per kilogram was given. A third injection of 30 cc. per kilogram was made with the result that there was a gradual return to normal. The course of this experiment is shown in group 3, experiment C4. The four other animals that suffered loss of the same amount of blood, except C2 which was bled to the extent of 35 cc. per kilogram, were treated in the same way and showed an initial rise of pressure of 10 mm. as an average. This soon began to decline and repeated injections of saline solution were without effect.

Group III

EXPERIMENT	HAEMORRHAGE IN CUBIC CENTIMETERS PER KILOGRAM	5	10	15	20	25	30	35	40	SALINE IN CUBIC CENTIMETERS PER KILOGRAM	BLOOD PRESSURE
C1	Blood pressure, 125	120	111	102	92	76	60	44	26	50	35
C2	Blood pressure, 128	124	113	100	85	74	55	35		50	41
C3	Blood pressure, 127	126	119	109	100	90	77	57	23	50	0
C4	Blood pressure, 120	119	111	99	90	80	71	59	45	50	65
C5	Blood pressure, 135	132	120	107	95	83	67	53	37	50	50

In group 3 cats were used instead of rabbits. They were anaesthetized by the administration of ether by inhalation. The initial blood pressure was higher than in the rabbits, but the results are comparable with those obtained in the first two groups of experiments. At first rabbits were tried but they succumbed very quickly after the removal of 35 cc. or 40 cc. of blood per kilogram. Then cats were resorted to with slightly better results as has been recorded.

Pilcher and Sollmann explain the rise in blood pressure as due to stimulation of the vaso-motor centre causing vaso-constriction. They state that the vaso-motor centre is not affected by infusion of saline solution if the blood pressure be 60 mm. of mercury or above, but that when the blood pressure is below 60 mm. the vaso-motor centre may be stimulated by such injections (2). The author's results corroborate this assertion, at least so far as the influence of saline infusions when the blood pressure is below 60 mm. is concerned. No attempt was made to determine the effect of intravenous administration of saline solution at higher blood pressure levels as the experiments were undertaken for a different purpose. The object, as already stated, was to determine what could be hoped for in the way of restoring blood pressure and maintaining the circulation in cases of haemorrhage of varying degrees of severity. From these observations we draw the conclusion that so long as the haemorrhage has not been great enough to reduce blood pressure to the "shock level" gratifying results may be hoped for from the intravenous administration of normal salt solution. When the blood pressure has reached the level of shock, 30 mm. to 50 mm., restoration of blood pressure and maintenance of the vital functions of the organism are a possibility, but cannot be expected with any certainty. In general it can be stated that in haemorrhage injection of amounts of saline solution in excess of the amount of blood lost will give the best results; in severe cases the use of large amounts of normal salt solution, 50 cc. to 100 cc. per kilogram of body weight, is most likely to be attended by a successful outcome.

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STRUCTURE OF THE FIBRIN-GEL AND THEORIES OF GEL-FORMATION

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The usual view of the structure of the fibrin-gel is that it is composed of a fine reticulum of fibrin-threads, holding the water within its meshes. A reticulum of this character can be seen in fact with the microscope in a drop of coagulated blood as was first described by Ranvier, but recent observations demonstrate that this is not the primary mode of formation of the gel. This traditional fibrin net-work must be regarded as an artifact caused by mechanical tension or pressure exerted in the preparation. Stübel (1), making use of the dark field illumination discovered that in clotting the fibrin is deposited as separate needles or crystals. The author (2), who was at the same time studying the process of clotting with the ultramicroscope, making use of the slit-form, was able promptly to confirm and extend Stübel's discovery.[1] The object of the present paper is to report further observations upon the structure and formation of the fibrin-gel.

THE CRYSTALLINE GEL

The mode of formation of the fibrin needles was observed in most cases by using the clear cell-free plasma obtained by centrifugalizing oxalated mammalian blood. To specimens of this plasma thrombin, in saline solution, was added and the mixture was then introduced into the ultramicroscope cell for observation. The thrombin,

[1] Hekma (Internationales Zeitsch. f. physikalisch-chemische Biologie, 1915, 2, 354) calls attention to the fact that fibrin needles were observed many years ago by Schimmelbusch (Virchow's Archiv, 1885, 101, 201). He described them as very thin spindle-shaped needles, 5 to 20 micra long, which are formed independently of the corpuscular elements of the blood. His observations were overlooked apparently or were not confirmed by subsequent workers, but his figures show that he was dealing with the same structure whose existence is now revealed so easily and so unmistakably with the aid of the ultramicroscope.

prepared by a method previously described (3), was kept in dried condition, a small portion being dissolved for use when needed. The mixture of oxalated plasma and thrombin clotted in a shorter or longer time according to the concentration of thrombin used, and it was easy to choose such a concentration as would induce clotting in a convenient time for observation. The oxalated plasma when possible was obtained from fasting animals and was therefore free from noticeable amounts of fat. Under the ultramicroscope it showed scattered large particles, probably fat granules, and a luminous cone, marking the beam of light, in which no visible particles could be distinguished. The colloidal material in the plasma exists, therefore, in a degree of dispersion not resolvable by the ultramicroscope, the particles coming under the general designation of amicrons. The thrombin solutions, in the concentration employed, showed under the ultramicroscope a number of scattered large particles which presumably, on account of their small number, are to be considered as foreign material. For the rest the field was almost optically empty with a faint indication of the presence of a luminous cone.

The appearances observed, when the two solutions were mixed in proportions sufficient to cause clotting, varied according to conditions. If the amount of effective thrombin was for any reason insufficient to cause prompt or complete clotting one might observe first a certain increase in the luminosity of the opalescent cone, and later separate minute needles of fibrin appeared here and there in the field, coming into view quietly and floating slowly in one direction or another. If the clotting was imperfect these needles never became numerous enough to cohere into a solid mass. Under such circumstances the mixture when removed from the observation cell would show no visible clot. If, however, the amount of thrombin was sufficient to give a perceptible clot the formation of needles, once it had begun, would proceed steadily until they formed a meshwork of intermingled crystals. In mixtures that clotted firmly in from five to ten minutes the formation of the crystals began somewhat suddenly over the whole field and proceeded rapidly until the field was converted into a mass of the intermeshed needles. The intermediate steps in the formation of the needles were followed most successfully when the plasmas were much diluted with salt solution (sodium chloride 0.9 per cent) or when fibrinogen solutions were employed in place of the oxalated plasma. Under these conditions the steps that could be made out were as follows. First, an intensification of the luminosity of the opalescent cone, which we may

assume was due to a beginning aggregation of the amicrons into particles of larger size. Second, a granulation of the whole field. Brilliant shimmering particles appeared throughout the whole cone, and these particles exhibited not only the usual Brownian movements shown by particles of such size, but in many cases also abrupt almost jumping movements which took them into or out of focus and gave to the whole field the appearance of an agitated scintillating mass. The particles rapidly assumed the visible shape of small rods, like short bacilli, and then grew into the longer acicular crystals. With the increase in size the abrupt and the Brownian movements were diminished and the final picture was the mass of brilliant stationary needles closely intermeshed. The inference to be made from these appearances is that the fibrin needles are formed by the aggregation of the amicrons of the fibrinogen solutions, and that in this aggregation or precipitation under the influence of the thrombin a vectorial force is brought into play which controls the agglutination of the particles into definite crystal-like needles. Fibrinogen may be precipitated out of its solutions in various ways, by the action of heat, of neutral salts, dilute acids, etc., but in all such cases the fibrinogen particles are aggregated into amorphous clumps, whereas under the influence of thrombin the particles are arranged in accordance with a directive force which is developed in the interaction of the thrombin and the fibrinogen.

The deposition of fibrin needles when oxalated plasma is clotted with thrombin has been observed with hundreds of specimens of blood from human beings under normal and pathological conditions and from various mammals. An objection may be made possibly to the use of the isolated thrombin in these cases and some doubt may be raised as to whether or not the thrombin as it normally occurs in the blood has a similar action.

To meet this possible objection the following methods were employed to insure the clotting of the plasma by means of its own thrombin.

1. The blood as it flowed from the artery was received into a 20 per cent solution of sodium or potassium chloride in proportions to yield a mixture contain 5 per cent of the salt used. This concentration of salt prevents coagulation. The mixture may be centrifugalized to obtain a cell-free plasma and this plasma clots readily on simple dilution with water. Specimens of this kind observed under the ultramicroscope showed a typical deposition of fibrin-needles.

2. Oxalated and centrifugalized plasmas clot readily and firmly on

the addition of a suitable amount of calcium chloride. If the amount of calcium chloride is properly chosen the resulting precipitate of calcium oxalate may be centrifugalized off and the clear supernatant plasma may be introduced into an observation cell before clotting occurs. When the clot forms under these conditions it is accompanied by a dense deposition of fine needles.

3. Slow clotting bloods, such as that of the bird, may be centrifugalized to remove corpuscles, and the clear plasma which clots spontaneously after a long time, may be introduced into the cell for observation under the ultramicroscope. Here also the clot when it forms is found to consist of a meshwork of fibrin needles.

There can be no doubt therefore that the normal process of clotting, in the mammalian blood at least, results in the formation of needle-like crystals of fibrin, which are formed separately but which later become intermeshed and possibly fused together. The normal blood-clot is a crystalline gel. It is a matter of interest to inquire whether the clot shows this structure in the blood of all animals. I have examined the blood of several mammalia (man, dog, cat, rabbit, horse, pig), of the bird (hen), of the reptile (terrapin) and of the invertebrate, the crab.

Among the mammals it can be shown easily, by the methods given above, that normal clotting consists in a deposition of fibrin needles, but undoubtedly the process is more easily disturbed or altered in some bloods than in others. For example, when the plasma (oxalated and centrifugalized) of the cat's blood is diluted four times or more with saline (0.9 per cent sodium chloride) and then clotted with thrombin a structureless clot may be obtained in which no fibrin needles occur, particularly if the amount of thrombin is large enough to cause prompt clotting. With dog's blood or human blood on the contrary dilution even a hundredfold with saline causes no such effect; up to a high degree of dilution addition of thrombin causes the formation of needles, indeed the details of the process of formation of these needles can be seen more clearly in some respects when the plasma is highly diluted. In the blood of the bird and the terrapin some difficulty is found in inducing coagulation conveniently in the oxalated and cell-free plasmas. As is well known the bloods of these animals clot very slowly, when protected from any admixture with tissue-juice, owing, as the author believes, to the relatively large excess of antithrombin present. Owing to this excess of antithrombin the centrifugalized oxalated plasma of such bloods does not clot readily upon the addition of thrombin solutions, and the animal's own blood serum is even less satisfactory for

this purpose, since, unless perfectly fresh, it may contain no effective thrombin. The thrombin in the sera of these animals passes into the ineffective metathrombin stage much more quickly than in the case of mammalian blood. In a number of ways however it may be demonstrated that the reptilian and the avian blood like the mammalian blood gives fibrin crystals on clotting. The most conclusive proof that this constitutes the normal clot in these bloods is obtained by allowing them to clot spontaneously in the ultramicroscope chamber. By using paraffined canulas and receptacles the blood of the hen or terrapin can be drawn off and centrifugalized without clotting. The normal cell-free plasma may then be pipetted off, placed in the observation cell and allowed to clot spontaneously. Under such circumstances the clot shows a typical crystalline structure as in the case of mammalian blood. When oxalated bird's plasma was used, after centrifugalization, and the thrombin was added in the usual manner no clotting en masse was obtained owing to the large amount of antithrombin present. But although in such a specimen no visible gel was formed, nevertheless, in the ultramicroscope chamber it could be observed that separate fibrin needles were deposited. In accordance with the slowness with which the process occurred these needles were unusually long, but they were scanty in number and entirely separate floating slowly into and out of the field of vision. Fibrinogen prepared from the oxalated plasma of the bird or terrapin by the usual method of successive precipitations by half-saturation with sodium chloride gave a typical crystalline gel upon the addition of thrombin, provided at least two precipitations were made of the fibrinogen with the usual precautions of washing the precipitate. The fibrinogen obtained by a single precipitation of the plasma might fail to give a clot with thrombin, owing no doubt to the excess of antithrombin present in the original plasma and still contained in the first precipitate of fibrinogen. These results it may be noted give a striking proof of the non-specificity of thrombin. The thrombin used was prepared in all cases from the fibrin of pig's blood and it was effective in causing a typical crystalline gel with the plasma or the fibrinogen from any other mammalian blood or apparently with the blood of any vertebrate animal. It should be stated however that in the plasma of both the bird and the terrapin the fibrinogen can be altered or denatured more readily than in the case of the mammalian blood. Simple dilution of the plasma or, in the case of the bird, the presence of much fat in the blood seemed to alter the fibrinogen so that the gel produced by thrombin differed in its

physical properties and ultramicroscopic structure. Fibrinogen is an unstable protein which is readily denatured to a greater or less extent by variations in reaction and changes of other kinds, and it would seem that in this susceptibility to alteration in properties the fibrinogen of the bird and reptilian blood is more unstable than the similar protein occurring in mammalian blood.

While all of the vertebrate bloods examined give a crystalline gel when the conditions of clotting are normal this was not found to be the case in the blood of the crab. The blood of this animal gives a firm clot when removed from the body. If a specimen of the blood is taken from the heart by means of a syringe and transferred to the observation cell normal clotting occurs and the structure of the clot may be examined ultramicroscopically. Specimens obtained in this way showed no structure whatever. There were a few scattered large particles, but the blood before and after setting to a gel showed simply a luminous cone in which no visible particles could be made out. The gel was wholly structureless so far as the ultramicroscope could determine. Since the crab's blood gives an alkaline reaction it was thought, in accordance with the facts described in the next paragraph, that this might explain the lack of structure in the clot, and that in a neutral or slightly acid reaction fibrin needles similar to those of mammalian blood might be obtained. In one experiment therefore decinormal hydrochloric acid was added to the blood taken from the heart in the proportion of three drops of the acid to 1 cc. of the blood. The acid causes an abundant precipitate in which the corpuscles are entangled. By filtering a clear liquid is obtained which takes on a blue color and soon sets to a stiff non-retractile clot. Specimens of this plasma allowed to coagulate in the observation cell gave a clot which exhibited a fine granular structure, the particles showing some tendency to form short beaded threads. There was however no indication of the formation of fibrin needles such as are given by the vertebrate blood. That there is an essential difference in the properties of the fibrinogens of vertebrate and invertebrate (crab) blood is shown also by their reactions with thrombin. The blood of the vertebrates, so far as I have examined them, show little or no indication of a specificity in regard to thrombin. The oxalated plasma or the isolated fibrinogen of any of these bloods is readily clotted by the pig's thrombin, although in the case of the plasmas of the bird and the reptile it may be necessary to neutralize the excess of antithrombin by the addition of thromboplastic material (kephalin solution). Crab's blood or fibrinogen prepared from it by

the usual method is, on the contrary, wholly unaffected by the mammalian (pig) thrombin used in these experiments. The process of blood coagulation among the vertebrates is essentially the same throughout, although minor differences in the properties or reactions of the fibrin-factors may be demonstrated in the different members, but among the invertebrates, if we may generalize from the results with crab's blood, the fibrin factors are of a different character and the gel formed by their interaction has a different structure.

THE STRUCTURELESS GEL

Under certain conditions mammalian fibrinogen may be made to give a structureless gel with thrombin. The gel in these cases is characterized by an entire lack of structure under the ultramicroscope, by its transparent appearance, by a diminution in or entire lack of retractility and by its easier solubility in dilute acids. I have been able to obtain these structureless gels by several different methods which may be described briefly.

1. *By the action of alkalies.* Fibrinogen solutions that give a typical crystalline gel with thrombin may be made to give a structureless gel if treated with a dilute solution of sodium carbonate or sodium bicarbonate in amounts sufficient to give an alkaline reaction on the addition of phenolphthalein. If the amount of alkali added is too large or it is allowed to act for too long a time the fibrinogen may be so altered that it will fail to clot at all with thrombin, but with the proper degree of alkalinity the formation of a clear structureless, non-retractile clot may be obtained readily. The same alteration in the fibrinogen may be produced by adding sodium carbonate (Na_2CO_3 0.25 per cent) to oxalated blood plasma. In this case much more of the carbonate must be added to obtain a distinct alkaline reaction with the phenolphthalein. In both cases, whether the oxalated plasma or the fibrinogen solution is used, the fibrinogen may be restored to its original condition in which it yields a crystalline gel with thrombin by simply precipitating it from its solutions with dilute acid or strong solutions of sodium chloride and redissolving the precipitate in a dilute (1 per cent) solution of sodium chloride, in the latter case, or by the addition of a drop or two of weak alkali ($HNaCO_3$, 0.5 per cent) in the former case.

2. *By standing.* If the oxalated and centrifugalized plasma is allowed to stand, in a refrigerator, for several days the fibrinogen is altered so that on addition of thrombin a structureless gel is formed.

The time necessary for this change to occur varies with individual bloods, usually it requires from five to seven days, but it may take place, with cat's blood especially, in as short a time as seventy-two hours. As in the case of the alkali treatment prolonged standing results in a more complete denaturing of the fibrinogen so that it fails to clot at all with thrombin. In the intermediate stage in which it gives a structureless clot the fibrinogen of the plasma may be restored to its original normal condition by precipitation with neutral salts or weak acid and appropriate resolution.

3. *By dilution.* As mentioned above cat's plasma if diluted about fourfold with saline (sodium chloride 0.9 per cent) undergoes this alteration, especially if the proportion of thrombin used is large. Human and dog's plasmas are not affected in the same way by dilution. The fibrinogen precipitated from the cat's plasma is not altered in its properties by simple dilution.

4. *By drying.* The author has made frequent use of dried plasmas in his experiments. They are prepared by dialyzing the oxalated and centrifugalized plasma against a large volume of saline (sodium chloride 0.9 per cent) to get rid of the excess of oxalate, and then drying down the dialyzed plasma in small lots in watch glasses. The dried specimens are kept in a desiccator. They undergo a gradual deterioration with time. When freshly prepared they dissolve readily in saline solution and give a firm clot on the addition of thrombin. In fresh specimens the clot may show fibrin needles more or less perfectly formed. Later the clots pass into the structureless variety and in specimens that have been kept in the desiccator for many months the fibrinogen becomes so altered that it gives only an imperfect clot or none at all when thrombin is added. With the freshly prepared material it was often observed that solutions made with distilled water gave a crystalline gel with thrombin while those made with a solution of sodium chloride gave a structureless gel. The excess of sodium chloride seemed to act as a weak alkali. In this last case if the solution in sodium chloride was precipitated with weak acid and redissolved by the addition of weak alkali it would show fibrin needles when thrombin was added.

5. *By the administration of emetin chloride or of oxidized epinephrin.* In the course of experiments made by Drs. Levy and Rowntree (4) upon the effect of intravenous injections of emetin chloride it was noticed in some cases that the blood taken just before death not only showed delayed and imperfect coagulation but gave also a non-retractile

clot. Some of this blood was therefore oxalated and centrifugalized and the clear plasma was clotted with thrombin in the observation cell. In some cases fibrin needles more or less imperfect were obtained, while in other cases the clot showed no structure and these last cases were the ones in which the clot also exhibited loss of retractility.

Somewhat similar results were obtained in a series of experiments made with Mr. Sosman upon the effects of intravascular injections of oxidized epinephrin. While lethal doses of epinephrin had no uniform effect upon the coagulation of the blood, injections of a certain oxidation product caused a marked delay in the time of coagulation and in some cases so changed the fibrinogen that a structureless instead of a crystalline clot was obtained by adding thrombin to the oxalated plasma.

In both of these cases of intoxication the blood, as tested with neutral red, gave indications of the development of a condition of acidosis, but this condition could not have caused directly the change in fibrinogen. Acidosis produced in other ways was not attended by any alteration in the structure of the clot.

These observations were of a more or less incidental character and were not extended by experiments with other organic bases, but they suggested the possibility that in some pathological conditions in man a similar change in the character of the clot might occur. With this idea in mind observations were made upon the blood of a great variety of patients in the Johns Hopkins Hospital. The blood was collected by venepuncture, oxalated and centrifugalized, and the clear plasma was then clotted in the observation cell by the addition of a solution of thrombin. The results need not be described in detail as they were wholly negative. In all the pathological bloods examined so far, including cases of pernicious anemia, cardiac and renal cases, typhoids, pneumonias, hemophilics, purpurics, secondary anemias, etc., the blood plasma on clotting gave a typical crystalline gel.

Of the several methods enumerated above by means of which the fibrinogen was so altered as to give a structureless gel with thrombin the simplest is the first in which the hydroxyl-ion concentration was increased. It is possible that in the other methods the same end result was obtained. An increase in hydrogen-ion concentration leads to an increasing aggregation and finally a precipitation of the colloidal particles of fibrinogen. Thrombin added at any time short of the actual precipitation gives fibrin needles and a crystalline gel. An increase in hydroxyl-ion concentration on the contrary causes a

greater degree of dispersion and stability in the solution of fibrinogen, the gel formation with thrombin requires a longer and longer time for its completion and finally fails entirely. At some point in this increasing alkalinity, approximately at a hydrogen-ion concentration of $H = 10^{-9}$ the fibrin needles fail to appear and the gel becomes structureless. In intermediate stages the needles lose their sharp outlines, take on the appearance of broken beaded filaments or short rows of granules, while the gel that is formed loses correspondingly its property of prompt retraction.

It is a matter of interest in connection with the problem of gel-formation to call attention to the fact that when alkali is added to a fibrinogen solution or a blood-plasma in amounts sufficient to prevent the thrombin from causing any visible clotting there may still be an effect in the direction of a marked increase in viscosity. That is to say the thrombin exerts an influence upon the fibrinogen which causes the latter to unite with or bind the water to some extent, as shown by the increase in viscosity, although for some reason, possibly an increased degree of dispersion, this action does not go far enough to form an actual gel. The following method was used to establish this fact.

Specimens were used of oxalated and centrifugalized plasma of the dog and of fibrinogen prepared from this plasma. Sodium carbonate was added to the fibrinogen solution and plasma in amounts sufficient to prevent the subsequent addition of thrombin from giving a visible clot of any kind. The viscosity of these solutions, with and without the addition of thrombin, was determined by the use of Hirsch and Beck viscosimeter tubes at a constant temperature and under the action of gravity. In all cases the addition of the thrombin caused a distinct increase in viscosity. As an example the following case may be cited.

Mixture A. Oxalated plasma, 2.5 cc.; sodium carbonate, 5 per cent, 0.5 cc.; thrombin solution, heated for 5 minutes at 80°C. to destroy its efficacy, 1 cc.

Mixture B. Oxalated plasma, 2.5 cc.; sodium carbonate, 5 per cent. 0.5 cc.; thrombin solution, 1 cc.

These mixtures were allowed to stand for 1 hour, 15 minutes, and the flow through the viscosimeter tubes was then measured at a temperature of 17.5°C. Time of flow for A = 69 seconds. Time of flow for B = 79 seconds.

The specimens were allowed to stand for an additional four hours and determinations were again made, the temperature of the bath being

19°C. Time of flow for A = 65 seconds; Time of flow for B = 77 seconds.

The retraction of the fibrin-gel. The retractility or contraction of the blood-clot is one of its best known and most important properties. Various observers have attempted to connect this property with the presence of blood-plates. Le Sourd and Pagniez (5) state that in the blood of an animal made plateless by immunization the clot loses its power of retraction. But that the plates have no necessary causal connection, mechanical or otherwise, with the retractility of the clot seems to be shown clearly by the fact that cell-free oxalated plasmas or solutions of pure fibrinogen made to clot by the addition of thrombin show this property to a marked degree. Its existence may be obscured by the adhesion of the clot to the walls of the containing vessel, but if the clot is loosened from the walls it quickly contracts forcing out a clear serum. In normal blood the plates may be connected indirectly with the phenomenon of contraction in that they serve as a source of thrombin and thromboplastic substance, and the firmness and retractility of the clot are increased with the concentration of these factors. The observations described above in regard to the differences in properties between the crystalline and the structureless gel seem to show that the phenomenon of contraction, the syneresis of the clot, is connected directly with the existence of the fibrin-needles. In the wholly structureless clot the gel is soft and transparent. It divides easily into pieces or fragments which may again flow together, but there is no indication of retraction or the formation of an expressed serum. The crystalline gel on the contrary shows always a marked tendency to contract. Even in very dilute solutions in which the fibrin is deposited as a delicate membrane, contraction is shown distinctly when the membrane is detached from the walls or is shaken gently; it shrivels up promptly to a much smaller membrane-like structure. It seems most probable that the contraction of the normal clot is an instance of the gradual change or aging of the colloided aggregate and is referable to a process of further condensation in the particles composing the needles. Contraction is a phenomenon exhibited by many gels, but certainly it is much more marked in the blood clot, especially of the mammal, than in the gel of gelatine, agar-agar or casein, or in the structureless gel of the crab's blood. The development in the vertebrates of a fibrinogen capable of yielding a crystalline retractile gel and the increasing perfection of this property in the higher vertebrates are explicable possibly in terms of a more perfect adaptation of this form of clotting to the prevention of hemorrhage.

CATAPHORESIS EXPERIMENTS

The precipitation of fibrinogen by thrombin suggests a reaction between oppositely charged colloids and in accordance with this suggestion a number of experiments were carried out to determine the electrical charges if any, carried by these substances. The device used was essentially that described by Michaelis (6). Connecting tubes were employed containing agar-agar made up in one per cent solution of sodium chloride as used by Field and Teague (7). See Figure 1.

A current of 110 volts was used with a current transmission varying from 1 to 10 milliamperes according as the outside tubes, 1 and 2, contained water or a solution of sodium chloride (0.9 per cent). The non-polarizable electrode on the positive side was made of silver immersed in a solution of sodium chloride to prevent the passage of the silver ions into the solution in tube 1. On the negative side the electrode combination was zinc and zinc sulphate.

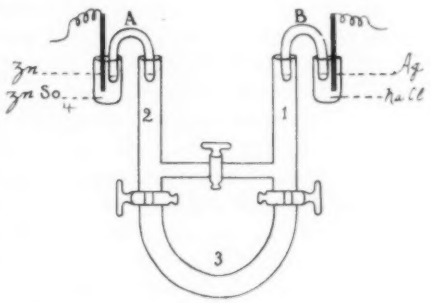

FIG. 1. Apparatus for Cataphoresis. Tubes 1 and 2 filled with water or with sodium chloride 0.9 per cent. 3 filled with the oxalated plasma. *A* and *B* connecting tubes filled with agar-agar made up in solution of sodium chloride 0.9 per cent.

So far as the thrombin is concerned it was found that in aqueous solutions of my purified thrombin, containing a little sodium chloride, some of the thrombin carried a positive charge. That is to say, after a cataphoresis lasting for an hour or more, with water in tubes 1 and 2, thrombin could be detected in tube 2 but was absent from tube 1. In serum, on the contrary, and in oxalated plasmas (prothrombin) that is, in slightly alkaline media, the thrombin and prothrombin exhibited a negative charge. They could be detected in tube 1 but not in tube 2. Since in such media the fibrinogen also exhibits a negative charge it would appear that the precipitation of the fibrinogen by the thrombin can not be referred to a reaction between oppositely charged particles.

Experiments of a similar character made upon oxalated blood-plasma after centrifugalizing off the corpuscles have yielded some sug-

gestive results. In these experiments the outside tubes 1 and 2 were filled with 0.9 per cent solution of sodium chloride and the current transmission varied from 4 to 5 milliamperes. With this arrangement it was frequently possible to effect a separation of the fibrinogen in the plasma, some of it going to the negative and some to the positive pole, indicating therefore the existence in the plasma of some positively charged and some negatively charged fibrinogen. This separation was obtained most certainly when the reaction of the plasma was brought to or toward the neutral point and the current was passed for a short time, about one hour. A drop of neutral red solution (0.25 per cent) was added to 10 cc. of the plasma and then $\frac{N}{10}$ HCl until the orange yellow color took on a distinct reddish tint. The two kinds of fibrinogen obtained from tubes 1 and 2 reacted quite differently to thrombin. The positively charged material that had accumulated in tube 2 gave with thrombin a flocculent precipitate which in some cases settled to the bottom, but in other cases adhered to form a membranous mass resembling a so-called membranous clot. In no case was there a formation of a gelatinous clot. Examined under the ultra-microscope the material in tube 2 showed very numerous coarse particles. Under the influence of the thrombin these particles agglutinated to form clumps or short strings in which the separate particles were distinctly visible. The thrombin in this case acted after the manner of an agglutinin. The negatively charged material that was carried over into tube 1 gave always with thrombin a gelatinous clot. Examined under the ultra-microscope this clot exhibited in some cases the presence of typical thrombin needles, but in other cases, especially with those specimens of plasma which had been neutralized with dilute acid, the clot showed only a few scattered short needles or rods. For the most part it was structureless or showed only faint indications of nebulous masses in which no particulate structure could be made out. Its characteristics in fact tended to approach those described above as the result of the initial action of alkalies on fibrinogen. The plasma remaining in tube 3 at the end of the experiment gave always with thrombin a typical crystalline gel. These results suggest that the fibrinogen particles or aggregates may adsorb both hydrogen and hydroxyl ions. Adsorption of hydrogen ions tends to cause the precipitation of the fibrinogen particles by increased aggregation. The aggregates thus formed are still further influenced by thrombin to agglutinate into larger masses, but they do not exhibit the property of gelatinization. The adsorption of hydroxyl ions tends to the formation of a structureless gel. The dif-

ference in reaction to thrombin exhibited by these oppositely charged fibrinogens suggests moreover an explanation of a peculiarity in clotting which has attracted the attention of many observers. In the clotting of solutions of fibrinogen with purified thrombin or in the clotting of specimens of plasma containing but little effective thrombin, in consequence for example of the presence of excess of antithrombin, it is noticed often that the clotting takes place in two stages. There is formed first a delicate membrane which on shaking quickly retracts to a small clump. Later a gelatinous clot forms. According to conditions the interval of time between the two stages may be brief or may amount to an hour or more. In terms of the suggestion made above it may be supposed that the first stage represents an action of thrombin on the fibrinogen particles that by adsorption of the hydrogen ions carry a positive charge, while the later gelatinization represents the slower reaction of the thrombin with the fibrinogen combined with the hydroxyl ions, in accordance with the general fact that the time of clotting is prolonged by an increase in alkalinity.

DISCUSSION

The formation of the gel. The peculiar gel formed by fibrin is an outspoken heterogenous system. The more solid phase, the fibrin-needles, is clearly separated from the liquid phase. But there is no indication at all of a merely mechanical inclusion of the water or external phase between more solid walls or septa such as is assumed in the net-work theory and especially the honeycomb theory of Bütschli. Hardy (8) has reported such a structure in gels of egg albumin, gelatine and India rubber, visible under the microscope as a solid framework holding liquid in the interstices. In a ternary system composed of water, gelatine and alcohol, or water, gelatine and corrosive sublimate he obtained a net-structure or honeycomb structure according to the concentrations used, and in a binary system of water and gelatine, 1.5 per cent, a similar honeycomb structure was observed when the solution was cooled to $-1°C$. This view seems to have prevailed generally. Freundlich for example in his "Kapillar Chemie" defines gels as diphasic systems consisting of very thin connecting walls of solid amorphous substance, enclosing spaces filled with liquid. But recent observers who have made use of the ultramicroscope have obtained no evidence of any structure of this kind. Bachmann (9), and Zsigmondy and Bachmann report their observations upon the

ultramicroscope study of the gels of silicic acid, agar-agar and gelatine. In dilute solutions of gelatine (1 to 6 per cent), while in a liquid condition, no definite structure can be seen. Outside certain optical impurities the field shows simply a cone of light due to the invisible amicrons. As gelatinization occurs there is a glimmering or sparkling movement in the cone resulting finally in the production of a swarm of minute submicrons. These submicrons are formed presumably by the aggregation or massing of the amicrons. When first formed they show translatory movements, but later only movements of oscillation that become less and less extensive until finally the particles are bound together in gelatine flocks which are stationary. The process is essentially the same as in the formation of the fibrin-gel described above, except for the appearance in the latter of the force which leads to the combination of the particles to form definite needles instead of amorphous flocculi. The important fact that remains to be explained is the binding of the water which leads to the solidification or gelatinization of the solution. As stated above the view that the water or more liquid phase is included between the walls of a solid phase, as in a sponge or honeycomb, finds no support at all in the results obtained from ultramicroscopic examination. Pauli (10) in an interesting paper has shown that in solutions of the emulsion colloids the viscosity increases with the ionization of the molecules. He assumes that in protein solutions the albumen ion undergoes hydration to a greater extent than the isoelectric molecules, hence the increased viscosity, and he suggests further that in gels we have simply an extension of this phenomenon, the solvent or disperse-medium being bound in great part as a hydrate. Obviously this theory of the binding of the water by the molecules or ions is not applicable to a heterogeneous system like the fibrin-gel. The fibrin-needles like other protein crystals possess probably the property of taking up water. Katz (11) has shown recently that in such cases the water is absorbed into the crystals in solid solution and not as a hydrate or as water of crystallization. But this process does not in any way explain the binding of the water surrounding the crystals. Other forms of crystalline gels are known. Zsigmondy and Bachmann (l. c.) have described such gels in the case of the soaps of the alkalies with the saturated or unsaturated fatty acids. As these solutions are cooled they form gels and the soaps separate out as needle crystals or fibers. Strong solutions of caffein are said to give a similar crystalline gel on cooling (Mathews). But the most complete analogy to the fibrin-gel is found in the interesting observations

published by Flade (12). If equivalent amounts of barium hydroxide and malonic acid are dissolved in methyl alcohol the addition of glycerine to the system causes the formation of a gel, slowly or quickly according to the concentrations used. The gel-formation is accompanied by the deposition of needle-crystals of barium malonate visible under the microscope but seen especially well with the ultramicroscope These crystals as pictured resemble very closely in form and arrangement the mesh of fibrin-needles obtained in the clotting of blood. The crystalline gel of barium malonate like that given by the alkaline soaps differs in one respect from the crystalline fibrin-gel. In the former we may assume that the crystals separate out from a saturated solution, and that in the liquid phase the solute is present in strong concentration. In the formation of a fibrin-gel on the contrary the fibrinogen will separate out completely from highly-dilute solutions. If one starts with pure solutions of fibrinogen none of the solute remains in solution in the liquid phase. In this case, therefore, it would seem to be necessary to conclude that the binding of the water can not be connected with any dispersed phase other than the fibrin-needles themselves. The surface energy at the interfaces between the crystals and the water-phase must be responsible for the solidification of the gel. Some authors who have taken this view refer the matter to the surface tension of the medium, which gives to the small water-films the properties of a solid. But it seems very doubtful whether this view offers an adequate explanation. Metallic sols of any degree of dispersion fail to exhibit the property of gelatinization. Very little seems to be known of the conditions of surface tension between the solid and liquid phases in such suspensoids. It is known that the surface tension at the water-air contact is not affected by the suspensoids, and it is to be presumed that in the hydrophobic colloids like the metallic sols the surface tension of the liquid phase at its contacts with the solid phase is greater than in the case of the hydrophilic colloids in which the separation of the two phases is less distinct. The property of gelatinization is however a characteristic of the hydrophilic and not of the hydrophobic colloids, and since in the latter the surface tension in the water films is probably greater than in the former this consideration would seem to exclude surface-tension of the water phase as the underlying cause of gelatinization. In accordance with this reasoning we are forced to seek for the cause of the solidity of the gel in the surface action of the solid phase. The fibrin needles bind the water by virtue of the molecular attraction or adhesion between their

surfaces and the water-molecules. This is presumably what Flade means when he states that the water in his gel of barium malonate is held by capillarity in the meshwork of crystals. From what has been said above in regard to the structureless fibrin-gel as compared with the crystalline gel it is evident that the gelatinization of the fibrin does not depend upon the existence of fibrin crystals. It may be assumed however that it does depend upon the existence of fibrin-aggregates. Under the influence of thrombin aggregates of this character are formed. Under certain conditions, neutral or weakly alkaline reaction, the aggregates unite to form the fibrin needles. In liquids of a stronger alkalinity the needles are not formed, but a gel is produced of a greater or less degree of firmness according to the hydroxyl concentration. The cause of the solidification of the blood-clot is to be sought in the special molecular attraction between the fibrin-aggregates and the water, and there seems to be no reason why this view should not apply to other gels such as those of silicic acid, gelatine and agar. It is in fact the view that was advocated years ago by Nägeli (13). "Die Micelle sich in Ketten an einander anhängen und ein Gerüste von Balken mit weiten Maschen bilden in welchem das wasser eingeschlossen ist und durch Molekularanziehung zwar nicht in einem ganz unbeweglichen aber doch in einem weniger beweglichen Zustande festgehalten wird." In the newer terminology we can substitute amicrons for "Micelle," and ultramicroscopic studies show that "Gerüste von Balken" are not a necesary structure of gels. The amicrons of the hydrophilic colloids may be massed or aggregated in amorphous or in crystalline forms. These aggregates in some cases cause gelatinization and in others do not. It is in the former group that we must suppose there exists a peculiar intensity of molecular attraction between the solid or internal phase and the water. As the name indicates all hydrophilic colloids exhibit this attraction for water and show a corresponding degree of viscosity so that as Höber expresses it the gels are not essentially different from hydrophilic colloid solutions. But in gels such as that formed by the fibrin-aggregates we must recognize a special degree in this attraction for water. The radius of the sphere of molecular action is large. The difference in this respect is illustrated especially well by fibrinogen solutions. When precipitated by acids or in other ways fibrinogen-aggregates are formed which eventually form large flocculi and settle out as a precipitate. When precipitated by thrombin, fibrin aggregates are formed which bind the water and form a gel.

The crystallization process and the nature of the reaction between fibrinogen and thrombin. The vectorial characteristic which causes the fibrin aggregates under normal conditions to assume the form of definite needles is dependent in some way upon an interaction between the thrombin and the fibrinogen. That is to say fibrinogen never aggregates out in this way except under the influence of thrombin and on the other hand even under the influence of thrombin the aggregation may not occur in crystalline forms if the fibrinogen is modified by increasing the hydroxyl-ion concentration. The way in which the thrombin and fibrinogen react is not known. According to the older view thrombin plays the part of a ferment or catalyst which initiates or accelerates a chemical change in the fibrinogen. But nothing is known regarding the difference in chemical structure, if any exits, between fibrinogen and fibrin. The evidence at hand would indicate on the contrary that thrombin does not act as an enzyme (14), but forms a compound with the fibrinogen. The process of formation of fibrin as seen under the ultramicroscope suggests that the needles are formed by a physical union of thrombin and fibrinogen particles, the whole process being one apparently of aggregation as in the case of the flocking due to precipitating reagents. If this is the case we should expect that the process might be reversed. That is to say conditions might arise under which the particles would be redispersed and the thrombin and fibrinogen be separated. As is well known thrombin can be obtained from thoroughly washed fibrin by digesting the latter in strong solutions of sodium chloride, and the fibrin when thus treated gives also a protein in solution which resembles fibrinogen in some respects, for example, in the temperature of heat coagulation. Hekma (15) claims to have shown that the fibrin-gel is reversible. An alkaline solution of the fibrin may be made to gel again by appropriate treatment, by the addition of acids for example. But since this result is obtained when the alkaline solutions are boiled it seems evident that the thrombin factor is excluded in the second gelatinization and the reversal that he describes is not a reversal of the process or processes which lead originally to the formation of the fibrin-gel. No fibrinogen is formed in his method of reversal.

The most significant fact brought out in this paper in regard to the formation of the fibrin-needles is the connection of the crystallization process with the reaction of the medium. With a certain degree of alkalinity of fibrinogen solutions no needles are formed although a gel may still be obtained with thrombin. With a stronger degree of alka-

linity no visible gel can be detected after the addition of thrombin but a distinct increase in viscosity may be demonstrated. The cataphoresis experiments described above indicate that under appropriate conditions part of the fibrinogen may carry a positive charge and part may carry a negative charge. The former exhibits only the phenomenon of aggregation or agglutination under the influence of the thrombin, the latter may show gelatinization without any visible aggregation of particles. The negative or positive charge exhibited by fibrinogen may be explained most easily on the assumption that it is due to electrical adsorption of hydroxyl or hydrogen ions. It is known that fibrinogen responds readily to changes in concentration of the hydrogen and hydroxyl ions in the solution. The former tend to favor the rapidity of the reaction with thrombin, the latter have a reverse effect. Under the normal conditions that prevail in the blood the thrombin has a double effect on fibrinogen. It causes, in the first place, an aggregation of the fibrinogen particles to form the fibrin needles and in the second place it sets up the process of gelatinization or binding of the water phase. In the cataphoresis experiments described these two effects of the thrombin are separated in a measure, the fibrinogen driven to the positive pole exhibits one, while that carried to the negative pole exhibits the other. As a provisional hypothesis one might assume that in neutral or slightly alkaline media the fibrinogen adsorbs or binds both hydrogen and hydroxyl ions, that the presence of the former furnishes an essential conditions for the aggregation into needles which takes place under the influence of the thrombin, while the existence of the hydroxyl combination, in connection with the presence of thrombin, is necessary to the development of the water-binding properties of the fibrin-aggregates. Under conditions such as the addition of excess of alkali it may be supposed that the fibrinogen particles exist in a higher degree of dispersion and exhibit adsorption of hydroxyl ions alone, and under these conditions thrombin causes only gel formation or increased viscosity, but is not capable of aggregating the particles into visible masses, either amorphous or crystalline in form.

CONCLUSIONS

1. In the clotting of mammalian blood the fibrin is deposited as needles. The needles are formed separately by an aggregation of fibrinogen particles. They vary in length from 10 to 30 microns and

form a close meshwork. The normal clot may be described as a crystalline gel.

2. A similar crystalline gel is formed in the normal clotting of the blood of other vertebrates. The blood of the invertebrates (crab) gives a structureless gel.

3. The fibrinogen of mammalian blood may be modified so that it gives a structureless instead of a crystalline gel with thrombin. The simplest method of effecting this modification is by increasing the alkalinity of the blood. If the increase in alkalinity passes a certain concentration addition of thrombin fails to cause gel-formation but may still produce a distinct increase in viscosity.

4. In media with the normal reaction of blood thrombin and prothrombin when submitted to cataphoresis exhibit a negative charge. Under the same conditions the fibrinogen in oxalated plasma shows mainly a negative charge, but frequently, especially when the plasma is nearly neutralized, a part of the fibrinogen exhibits a positive charge. The reaction to thrombin of the positively and negatively charged portions is different.

5. In the theoretical discussion it is shown that the ultramicroscopic picture of the fibrin-gel is not explicable in terms of a honeycomb theory, that is, of the inclusion of a liquid phase within solid septa. The gel-character or property is due, probably, not to surface tension in the liquid films between the needles, but to surface action of the fibrin-aggregates upon the water.

6. The vectorial characteristic of the fibrin-aggregates is connected with the reaction of the medium. The view is suggested that adsorption of both hydrogen and hydroxyl ions by the fibrinogen particles plays a determining part in the two effects of the action of thrombin, namely, the directive aggregation of the particles and the property of gelatinization.

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THE TENSION OF CARBON DIOXIDE AND THE PERCENTAGE SATURATION OF THE HAEMOGLOBIN IN THE VENOUS BLOOD AT REST AND AT WORK

The Regulation of the Circulation Rate

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I

Some observations on the minute volume of blood passing through the lungs of man at rest and at work were published from this laboratory a year ago and at about the same time a very exhaustive treatise on the same subject appeared from the Finsen Institute in Copenhagen. The author, Lindhard (1), gave curves of the blood-flow which agree very closely with those plotted by us. Very recently Newburgh and Means (2) have published similar observations on two other subjects, one of whom was normal and the other had a double aortic and double mitral disease. They state

> For the sake of comparison we have plotted the blood-flows of C. L. and J. H. M. in terms of oxygen absorption and have shown them together with those of Boothby. The curves for blood-flow of the three subjects are very nearly coincident, which we believe is a fact of considerable importance in that it shows that the increase in the blood-flow is governed by the same law in different individuals, and is especially interesting since one of the three individuals observed had a badly damaged heart.

In the original paper (3) we showed that it was possible to construct from the data there given the following curves: (1) the blood-flow per minute; (2) the pulse rate; (3) total ventilation; (4) volume of blood per pulse beat; (5) percentage saturation of the haemoglobin in the mixed venous blood; (6) alveolar carbon dioxide tension; (7) respiratory quotient; (8) tension of carbon dioxide in the venous blood, allowing for the influence of the percentage saturation of the haemoglobin with oxygen; (9) hydrogen ion concentration of the arterial blood; (10) the

tension of oxygen in the venous blood, allowing for the total acidity of the blood; and finally (11) Henderson's oxygen pulse. These curves are reproduced in this paper in figure I.

Curve VIII in figure I, representing the tension of carbon dioxide in the venous blood, was plotted after making the proper correction for the influence of the percentage saturation of the haemoglobin with oxygen. It is, of course, possible to plot the carbon dioxide curve without making this correction. This would then represent the carbon dioxide tension of the blood if the haemoglobin were completely saturated with oxygen, as is the case when the carbon dioxide tension is determined by the direct experimental method (Curve XII).

If direct determinations of the uncorrected carbon dioxide tension and oxygen tension[1] are made for venous blood and found to agree with the calculated values, a very convincing proof would be presented to substantiate the data of the original paper and the method of calculation adopted for determining the secondary curves.

II

The method adopted by us for determining the carbon dioxide and oxygen tensions in the venous blood is the one recently described by Christiansen, Douglas, and Haldane (4) and consists in using the lungs as an aerotonometer on the principle introduced by Pflüger. The technic is as follows: After a maximal expiration a mixture of carbon dioxide and air enriched with oxygen is inhaled from a Krogh recording spirometer; the breath is held about five seconds and then an expiration made to the "Mittellage" to obtain the first alveolar air sample; then the breath is held as long as possible and a maximal expiration made at the end of which the second alveolar air sample is taken. If the inspired mixture is of such a composition that when diluted with the residual air the carbon dioxide tension or the oxygen tension is within 3 or 4 mm. of the actual venous tension, it is reasonable to assume that after holding the breath twenty seconds longer (at rest) the air in the lungs will be in final equilibrium with the carbon dioxide or oxygen tension in the venous blood.

The determination of the carbon dioxide tension. The percentage saturation of the haemoglobin with oxygen influences the carbon dioxide

[1] If the oxygen tension of the venous blood is known, the percentage saturation of the haemoglobin can be obtained from the dissociation curve of oxyhaemoglobin, making allowance for the total acidity of the blood.

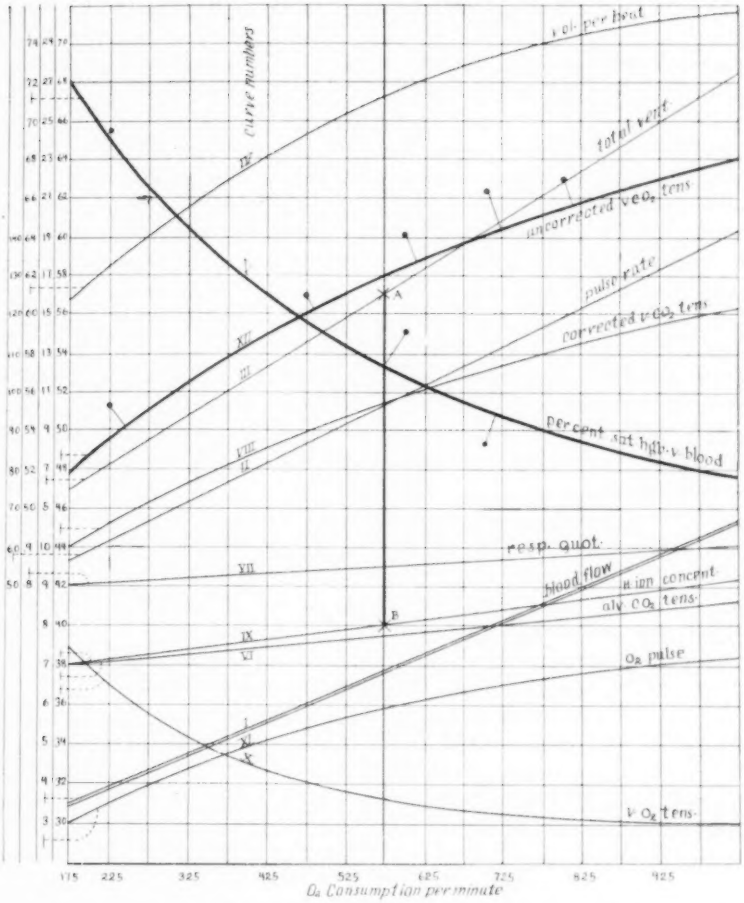

FIG. 1. Curves I to XI inclusive are the same as in our preceding paper (3). Curve I is the blood-flow per minute. Curve II, the pulse rate. Curve III, total ventilation per minute. Curve IV, volume per pulse beat. *Curve V, the percentage saturation of the haemoglobin in the mixed venous blood; connected to this curve are the points from Table I of this paper.* Curve VI, alveolar CO_2 tension. Curve VII, respiratory quotient. Curve VIII, tension of CO_2 in the venous blood, allowing for the influence of the percentage saturation of the haemoglobin with oxygen. Curve IX, hydrogen ion concentration of the arterial blood. Curve X, tension of oxygen in the venous blood allowing for the total acidity. Curve XI, oxygen pulse in cubic centimeters (Henderson). *Curve XII, uncorrected venous CO_2 tension determined from the nitrous oxide experiments; to this curve are connected the corresponding points given in Table I of this paper.*

dissociation curve; consequently a large excess of oxygen must be present in the inspired mixture to insure complete saturation of the haemoglobin with oxygen. For experiments at rest a mixture was made containing about 0.5 liters carbon dioxide, 1.5 liters oxygen and 4.0 liters air. For experiments at work slightly more carbon dioxide was used corresponding to the degree of work. If the carbon dioxide percentage is too high, it is impossible to hold the breath for a sufficient length of time to establish equilibrium between the venous blood and the alveolar air; if too low, not enough carbon dioxide is brought back to the lungs by the blood during the experimental period to produce such an equilibrium; and finally, if the oxygen percentage in the lungs is too low, the haemoglobin will not be completely saturated. Therefore it is necessary to take great care to prepare a mixture in the spirometer that will, when diluted with the residual air, produce a carbon dioxide tension in the alveolar air within 2 or 3 mm. of the carbon dioxide tension in the venous blood.

The determination of the oxygen tension. The method of determining the oxygen tension is similar to that of the carbon dioxide tension, except that the inspired mixture must be made up with a very large proportion of nitrogen. For experiments at rest the dead space in the Krogh spirometer was washed out with nitrogen and from 0.5 to 1 liter of air introduced; nitrogen was then added making a total of 6 liters. For experiments at work pure nitrogen was used.

We have made a large number of determinations of the venous oxygen tension at rest and consequently feel that the accidental errors have practically disappeared in the final average. For the experiments at work the results are less satisfactory as breathing pure nitrogen was quite dangerous and therefore we performed only a limited number of experiments and were satisfied in obtaining the general trend of the curve.

Instead of plotting the venous oxygen tension directly we have transposed it into terms of percentage saturation of haemoglobin with oxygen by means of the dissociation curve of oxyhaemoglobin given in figure 4 of the previous paper. This latter form of expression makes allowance for the variation in the carbon dioxide tension in the blood due to the method of obtaining the oxygen tension and is, therefore, the better way of representing the amount of oxygen in the venous blood.

As the tension of carbon dioxide and the percentage saturation of the haemoglobin in the venous blood vary with the amount of oxygen absorption per minute, a condition of body equilibrium must be estab-

lished. Experience has shown that this requires a preliminary period of about one-half hour during which time the subject rests or works at the same level as in the experiment proper. To obtain the oxygen consumption per minute a complete respiratory exchange experiment precedes the experiment proper.

For details of experimentation and calculation of results omitted in this communication, the reader is referred to our earlier paper.

II

In all we have performed two hundred and twelve experiments on the direct determination of the uncorrected carbon dioxide and oxygen tensions of the venous blood at rest and at work. The results are averaged into groups and presented in condensed form in the following table—Table I.

TABLE I

OXYGEN ABSORPTION	AVERAGE UNCORRECTED VENOUS CO_2 TENSION	NUMBER EXPERIMENTS IN AVERAGE	AVERAGE DEVIATION FROM MEAN	PERCENTAGE SATURATION HAEMOGLOBIN	NUMBER EXPERIMENTS IN AVERAGE	AVERAGE DEVIATION FROM MEAN
cc.	*mm.*		*mm.*	*per cent*		*per cent*
225	51.5	68	1.8	69.5	89	2.6
473	57.0	12	1.8			
604	60.1	8	3.1	59.3	6	3.8
705	62.4	10	4.4	53.4	13	7.6
800	63.1	6	1.9			

The average figures given in Table I are plotted in figure I. The large black dots represent the points determined by the present experiments and they are connected by a line to the corresponding curve calculated from the data of the previous investigation.

The direct determinations of the uncorrected venous carbon dioxide tension are seen to fall on a line parallel to but from 1.25 to 1.75 mm. higher than the curve calculated from the data of the previous experiments (Curve XII).

As these points stand they are an exceedingly strong confirmation of the previous data and calculations. The slight discrepancy in the two values is, on close examination, a further confirmation of the accuracy of the methods. In both there is a constant error due to the assumption that no blood makes a complete circuit during the time of the experiment. It was pointed out in the previous paper that the con-

stant error due to the presence in the lungs of blood that was making its second circuit was of an unknown but probably very slight order. By the nitrous oxide method the error from this assumption will cause the calculated tension to be slightly too low; in the present series, the same constant error will cause the tension determined directly to be correspondingly too high. Therefore, the true uncorrected venous carbon dioxide tension will be a mean between the curve calculated from the nitrous oxide experiments and that drawn through the points presented in this paper. The order of the error due to the recirculation of part of the blood during the time of an experiment is, then, in the region of 0.75 mm. for the uncorrected carbon dioxide tension.

The point indicating the percentage saturation of the haemoglobin with oxygen in the venous blood at rest represents the average of eighty-nine experiments. It lies within 0.5 per cent of the curve calculated from the nitrous oxide experiments and is therefore another strong confirmation of the accuracy of both methods.

The two averages obtained for the percentage saturation of the haemoglobin at work were from only a few experiments. They fall, however, about 2 per cent from the calculated curve, one above and the other below it. They show the trend of the curve but do not establish its position with the exactness of the uncorrected venous carbon dioxide curve (Curve V).

As pointed out by Christiansen, Douglas, and Haldane, it is possible from the above data to calculate the volume of blood passing through the lungs and it is obvious that essentially the same results would be obtained from these experiments as we found by the nitrous oxide method.

The fact that there is such close agreement in the data obtained by two entirely different methods is a very convincing proof of the accuracy of both methods for determining and calculating the circulation rate, the carbon dioxide tension and the percentage saturation of the haemoglobin in the venous blood.

It is evident therefore, that the circulation rate increases under conditions of work with the oxygen consumption in a manner corresponding to the increase in the total ventilation. We previously suggested that the actual activating substance which increases both the circulation rate and the total ventilation is the hydrogen ion concentration of the arterial blood. The fact, however, that an increase in the total ventilation can be very great when breathing carbon dioxide at rest without a corresponding increase in the circulation rate, as evidenced

by a proportionate increase in the pulse rate, suggests the possibility that the ventilation center is more sensitive to an increase in the carbon dioxide component and the circulation center to an increase in the non-volatile acid radicals composing the total acidity.

SUMMARY

1. The results are reported of two hundred and twelve experiments on the direct determination of the uncorrected carbon dioxide tension and percentage saturation of the haemoglobin in the venous blood at rest and at work.

2. The averages for various oxygen consumptions of the uncorrected venous carbon dioxide tensions fall on a line parallel to but from 1.25 to 1.75 mm. higher than the curve calculated from the experimental data obtained in determinations of the circulation rate by the nitrous oxide method, previously reported by Boothby.

3. The discrepancy in the two curves is caused by the error from the recirculation of the blood working in opposite directions in the two methods.

4. The average of the determinations of the percentage saturation of the haemoglobin in the venous blood at rest lies within 0.5 per cent of the curve calculated from the nitrous oxide experiments. One of the two averages for the percentage saturation at work falls 2 per cent above and the other 2 per cent below the calculated curve.

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(4) Christiansen, Douglas and Haldane: Journ. Physiol., 1914, xlviii, 4.

ON THE DETERMINATION OF CHARACTER AND QUANTITY OF THE RESPIRATORY CHANGE OF ARTERIAL PRESSURE IN MAN BY MEANS OF THE KOROTKOFF SOUNDS

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Among the questions that may be answered from the data to be had from routine indirect arterial blood pressure determinations on man there are two of considerable interest. These are (1) What is the character of blood pressure change during a respiration, does the pressure rise or fall during inspiration? (2) How much in terms of mm. of Hg. may the pressure be made to rise and fall during a respiration?

If Erlanger's sphygmomanometer is used the answer to the first question may be given by simultaneous observation of the cardio-respiratory waves in the sphygmomanometer tracing and the breathing movements (1). To do this well, however, a simultaneous tracing of the respiratory movements should also be taken as shown by Erlanger and Festerling (2) and as one of the present authors did in an extension of that work (8). Lewis (7) used a somewhat different method to determine the phase of respiratory rise of pressure, namely, by noting the phase of respiration during which the pulse-waves pass through the radial artery when a high systolic pressure is applied to the brachial artery. For this a "suspended sphygmograph" was used over the wrist.

In what immediately follows it will be shown that the Korotkoff sounds alone may be used for the determination of the first question put above. It will then be shown that by means of the Korotkoff sounds one may also measure the extent of blood pressure oscillation during the respirations.

During a study "on the inversion of the respiratory wave" in the blood pressure trace of man (8) it was observed that the Korotkoff sound when the cuff pressure was set in the vicinity of systolic or diastolic pressure is not heard with every pulse-wave. The sound seemed to become audible for only a part of the respiration, if one noticed the

respiratory movements. By having the subject breathe deeply and slowly the intermittent character of the sounds became so pronounced that the phenomenon was observed without difficulty. A practiced ear, however, could note the phenomenon even when the subject breathed normally.

A systematic exploration of this periodicity of the Korotkoff sounds soon showed that if the cuff-pressure was gradually lowered from systolic toward mean blood-pressure the period of silence during the respiratory cycle grew correspondingly shorter and the period of sounds correspondingly longer until a sound was heard (as usually observed) for every pulse-beat of the whole respiratory act.

It was recognized that this periodicity of the Korotkoff sounds is due to the fact that the levels of systolic and diastolic pressure themselves ebb and flow within the interval of a respiration.[1] And so with cuff-pressure set at mean systolic pressure a sound ought to be heard for every pulse-beat only during that portion of the respiration in which the (internal) systolic pressure is equal to or more than the (external) cuff-pressure. On the other hand, during that part of the respiration alone in which the (internal) systolic pressure is less than the (external) cuff-pressure the pulse-beats would be unaccompanied by sounds.

Furthermore under these conditions, and given that the inspirations are equal in time with the expirations, the period of sounds and the period of silence ought each to cover about one-half of the whole respiratory cycle. As the cuff-pressure is set above or below mean systolic pressure the ratios of the time intervals of sounds and silence ought to decrease or increase correspondingly, until the one or the other entirely vanishes.

If this explanation is correct for the periodicity of sounds with cuff-pressure in the region of systolic pressure then also one ought to observe for similar reasons, it was argued, a periodicity of sounds with cuff-pressure set in the region of diastolic pressure. Only in this case sounds would be heard with pulse-beats during that part of respiration alone in which the (internal) diastolic pressure falls below the (external) cuff-pressure; failure of sounds would cover that period of the respiration only during which the diastolic pressure remains above the level of cuff-pressure. With the latter again set at mean diastolic pres-

[1] By means of a sphygmograph attached to the wrist Lewis observed a similar periodicity of pulse-waves reaching the radial artery, when cuff-pressure was at systolic or high systolic pressure.

sure the two periods, one of sounds and one of silence, during a single respiration ought to be of equal duration.

While the periodicity of sounds in the two critical regions of blood pressure may thus yield pictures of similar character, there will be a common feature of striking difference. This difference ought to be one of right and left-handedness, or the difference of an object and its reflected image. With cuff-pressure at level of diastolic pressure the period of sounds ought to fall just in that part of the respiratory cycle which, when cuff-pressure is at level of systolic pressure, is covered by the period of silence, and *vice versa*.

Experiment showed that this is the case, as will be seen in the record below (fig. 1), and it may be pointed out here that this reversal of the periodicity of Korotkoff sounds in relation to the two phases of the respiratory act is only another expression of the "inversion of the respiratory wave in the blood pressure trace of man" to which reference has already been made (8).

THE DETERMINATION OF THE CHARACTER OF THE RESPIRATORY CHANGE OF PRESSURE LEVELS

It becomes clear now that we have a simple and direct means of determining whether rise of blood pressure accompanies the inspiratory or the expiratory phase of respiration. One need only set the cuff-pressure in the region of mean systolic pressure, have the subject breathe deeply and slowly, and observe simultaneously the periodic sounds and the phases of the respiration. If the period of sounds is heard during or at end of inspiration, or the period of silence occurs during or at end of expiration, the subject has *inspiratory* rise and expiratory fall of pressure. On the other hand, if the period of silence falls in with the act of inspiration and the period of sounds with the act of expiration, the subject has clearly an *expiratory rise* and inspiratory fall of arterial pressure. It may be said at once that we have observed both these kinds of respiratory change of pressure. The cardio-respiratory wave characterized by inspiratory rise, however, has been the more common among our cases, mostly young men. It has further been observed that the cases of inspiratory rise are also cases of inspiratory acceleration of heart-rate and apparently fall among the type showing the "young-heart" of Mackenzie, that is, those having labile vagal centres as discussed by one of us in an earlier paper (5).

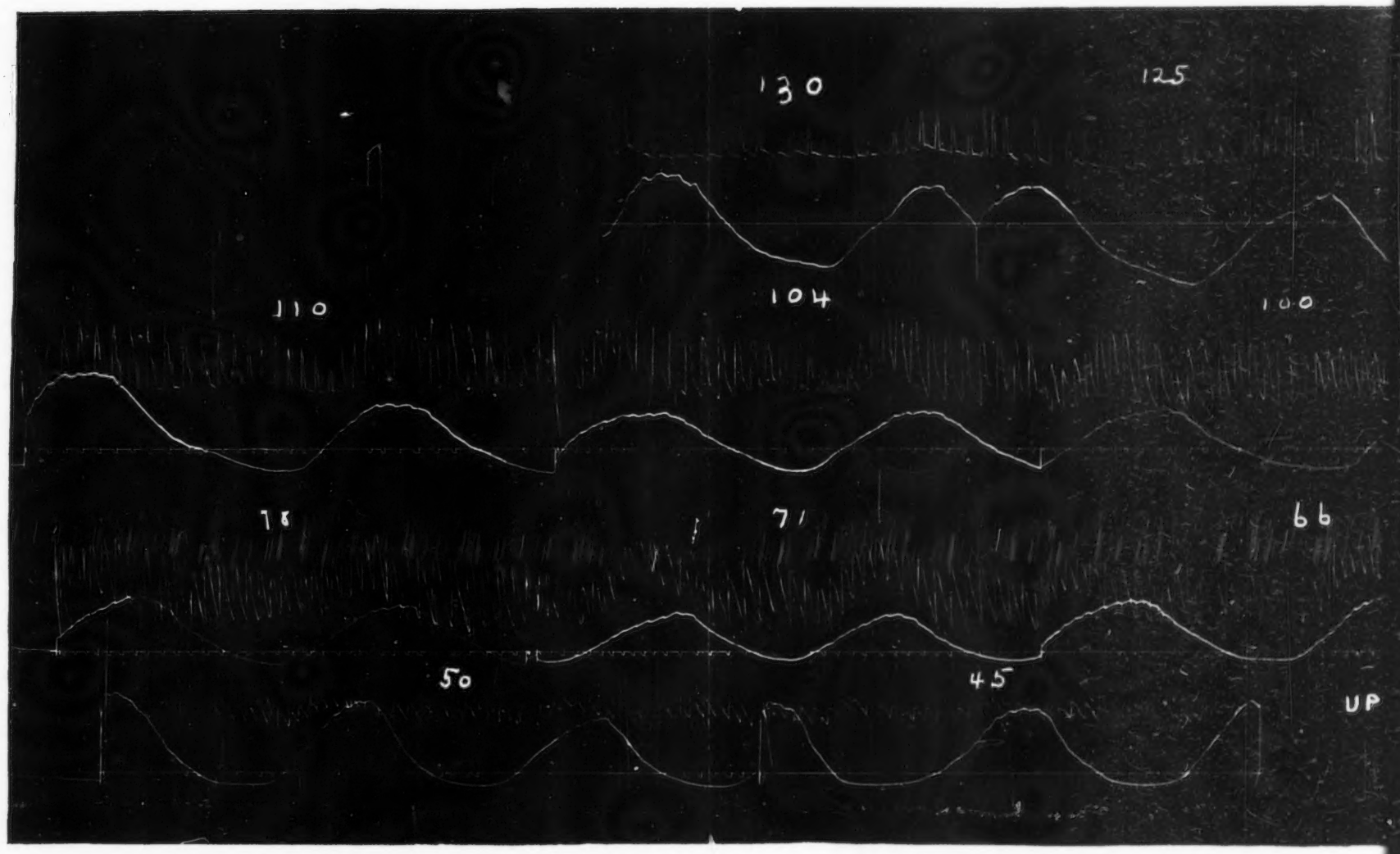

Figure 1. For explanatory note see

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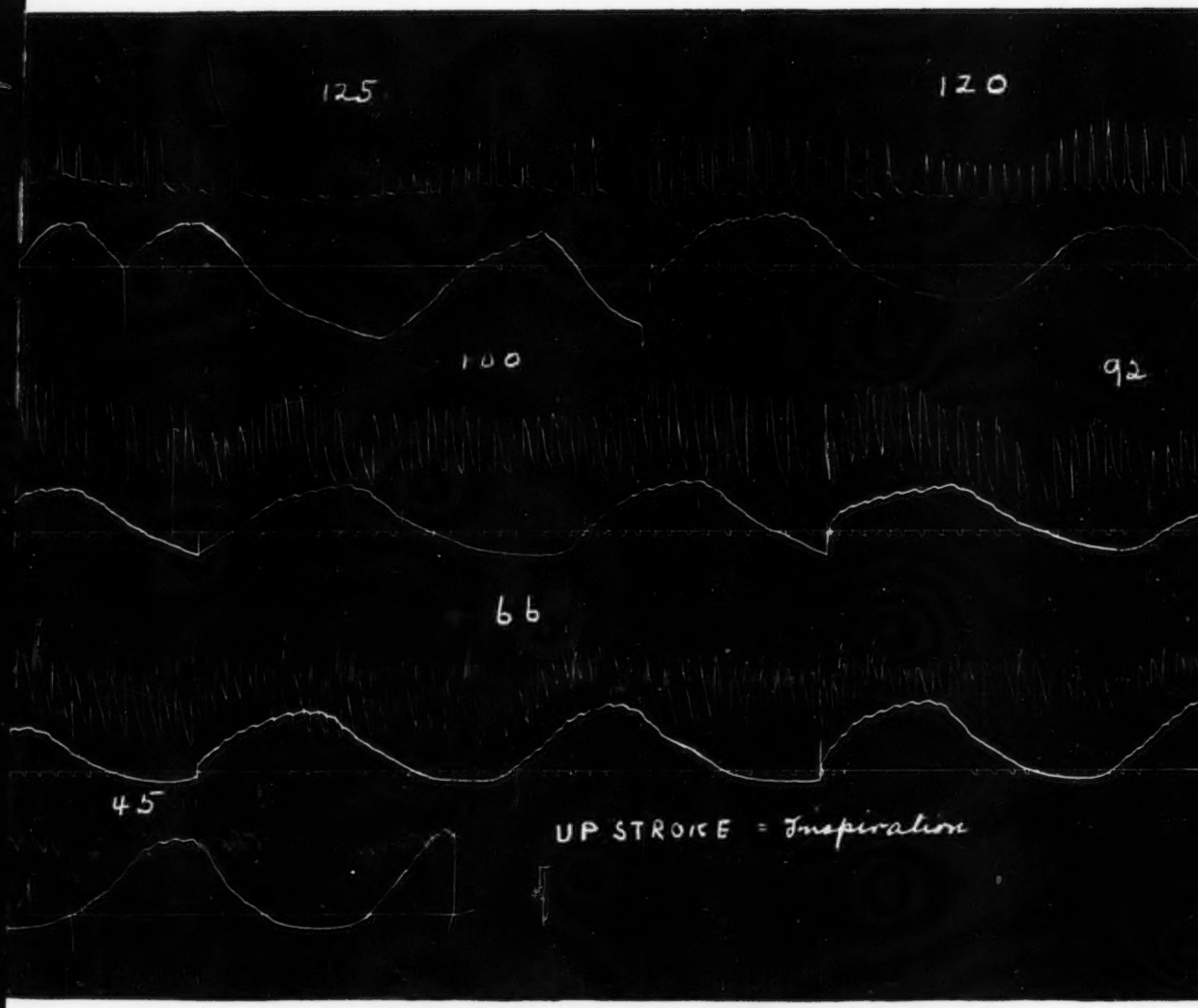

Figure 1. For explanatory note see page 565 of text

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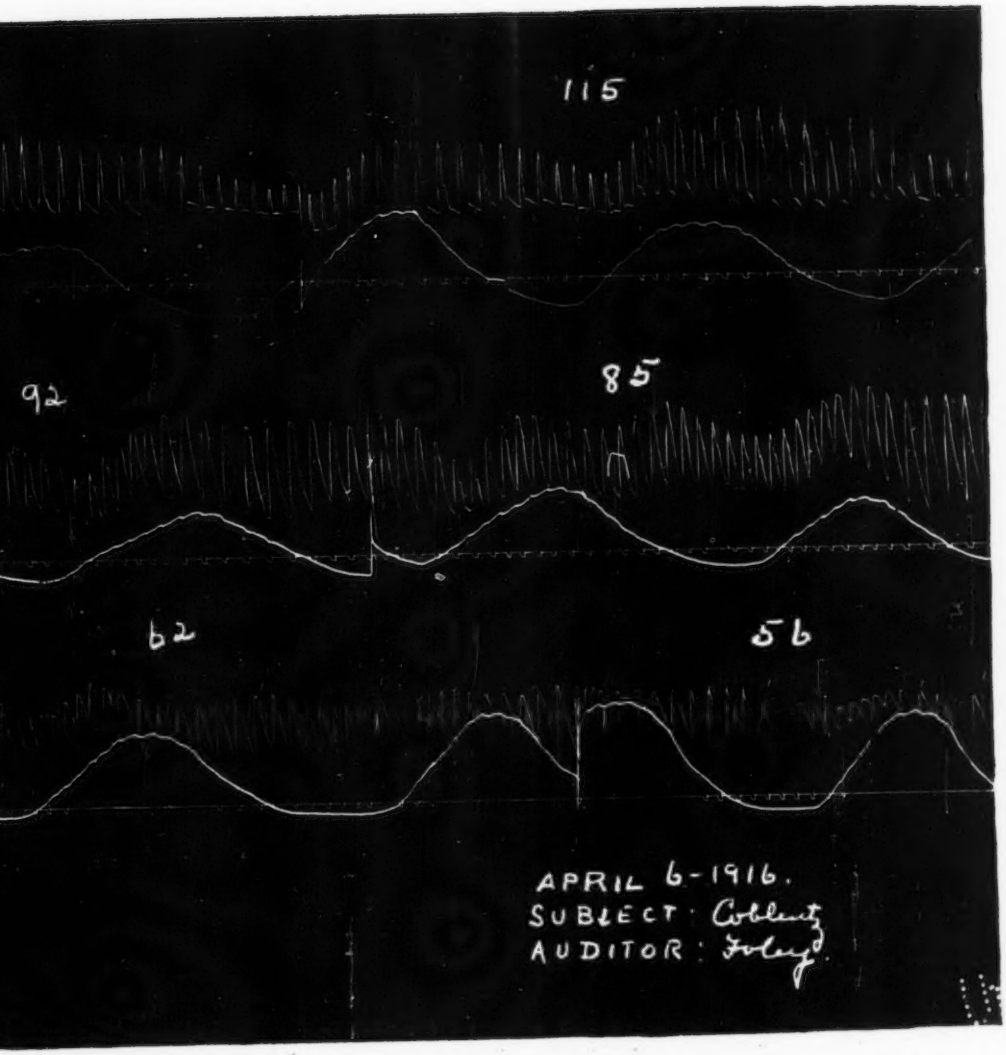

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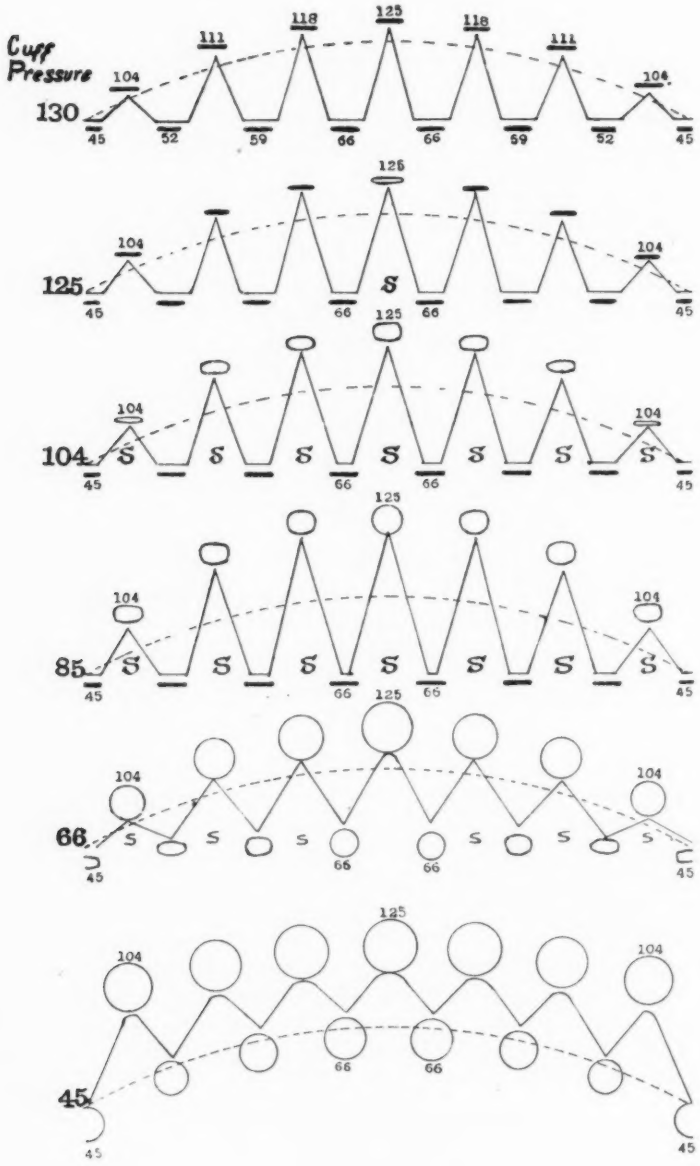
THE RELATION OF THE KOROTKOFF SOUNDS TO THE CRITICAL BLOOD PRESSURES IN MAN

As the reader doubtless has noted already, the principal argument in this paper depends upon the correctness of the view that the first and last Korotkoff sounds are true indices of systolic and diastolic pressures. As to the systolic index there is general agreement. That the last sound indicates diastolic pressure, however, is still in dispute. Since the *identity* of the first and last sounds with the critical pressures is the major premise in the argument, it makes it necessary for us to state what views we hold as to the conditions under which the sounds are produced. Inasmuch as other authors have already begun a systematic study of the causes of these sounds,[2] we shall make our discussion as brief as possible. One need not discuss the ultimate physical causes of the sounds heard over the compressed artery. The "water hammer" effect of Erlanger (5) appears to be very plausible and there seems to be very good ground for the necessary "half flattening" of the artery as brought forth by MacWilliams and Melvin (10). The views advanced by all these authors are freely drawn upon in what follows.

Evidence that the artery is completely flattened is obtained (if one has a delicate instrument) from the sphygmomanometer tracing itself. This becomes clear from the following consideration: In the ordinary sphygmomanometer tracing the individual pulse waves are made up of an upward excursion of the writing lever with systole due to an increasing arterial volume and a downward excursion with diastole due to a decreasing volume. Should the external pressure upon the artery be sufficient to cause it to collapse during a diastolic phase, a further fall of pressure in the artery (in more complete diastole) can impart no further change in volume to the cuff, for the volume of the compressed artery is already at a minimum. Therefore when the artery collapses for a certain period in the diastole one ought to find it indicated upon the tracing by a horizontal line marking the trough (diastole) of the pulse wave. For this horizontal line must indicate a constant arterial minimal volume and can be due only to obliteration of the artery. Otherwise the constantly changing arterial volume would make it impossible for the lever to remain at the same level for a period of time. In the same way when no horizontal is found in the trough of the pulse wave no constant volume is struck in diastole and the ar-

[2] See principally Erlanger (4, 5) and Brooks and Luckhardt (6)


Cuff Pressure
130
125
104
85
66
45
104
111
118
125
118
111
104
45
52
59
66
66
59
52
45

Explanatory note to figure 2

1 The diagram represents the principal blood-pressure events occurring within six respiratory cycles during a deep breathing (decompression) experiment.

2. Each cycle is shown with cuff pressure at a different level of pressure, the amount of which is indicated in the large numerals along the left of the diagram.

3. Each respiratory cycle moreover contains seven smaller waves representing the pulse waves of a sphygmomanometer trace, the crests of the waves being heights of systole, the troughs depths of diastole. The long dotted line indicates phases of the respiration, the upstroke being inspiration.

4. At the crest of each systole is figured the relative form and size of a cross section of the artery under the cuff for that particular systole; below the trough is figured the artery's lumen for that particular diastole. If complete obliteration obtains the lumen is indicated merely by a heavy dash.

5. The small numerals along the crests of the pulse-waves indicate the internal arterial pressure at height of the corresponding systoles. The small numerals below the troughs of these waves indicate corresponding diastolic pressures.

6. The pulse waves producing Korotkoff sounds are so indicated by a letter *S*, a large letter for the louder sounds, a small letter for the softer sounds.

7. At 130 mm. cuff pressure no sounds occur and complete obliteration of the artery prevails.

At 125 mm. cuff pressure one sound only is heard and the first patent lumen appears—"period of sounds."

At 104 mm. cuff pressure all pulse waves produce sounds and the lumens alternate between complete and partially flattened contours.

At 85 mm. cuff pressure all diastoles have obliterated lumens and one systole is able to change the lumen to a full circular shape.

At 66 mm. cuff pressure the first sound drops out—"period of silence." Other pulse waves produce the softer sounds, the lumens alternating between circular and partially flattened contours. The wave failing to produce a sound has the lumens alternating between contours of circles only, a condition which prevails for all pulse waves, with total absence of sounds, when cuff pressure is lowered to 45 mm. pressure.

8. In the sections of the diagram with cuff pressure set at 104 mm. Hg. or less an attempt is made to indicate roughly the portion of diastolic and systolic period during which the artery is completely obliterated. This is done by varying the length of the horizontal line in the trough of the pulse wave. As will be seen the flat troughs disappear entirely with cuff pressure as low as 66 and 45. Had this plan been carried out in the scheme with cuff pressures at 130 and 125 the pulse waves would all, save one, be perpendicular with the two limbs of each wave superimposed and the flat troughs covering the whole of the systole as well as diastole. These changes in the flattening of trough in the pulse waves can well be seen in sphygmomamometer tracings.

tery must not have been collapsed at any time during the cardiac cycle in question.

We are not to infer that when the external pressure is insufficient to cause collapse of the artery there is no deformation of the arterial cross section during diastole. It has been shown (MacWilliams and Melvin) that the artery undergoes a phase of partial flattening with lowered external pressures. Finally the external pressure may be lowered to such an extent that there is no deformation of the arterial crosssection even during complete diastole. Under these conditions the artery is in the same condition as regards its contour (but not as regards its diameter) as it would be were the cuff and external pressure absent altogether.

By inspection of the record (fig. 1) this sequence of events in regard to the character of the pulse wave is seen graphically recorded. At the pressure 85 mm. Hg. it is seen that the pulse tracing coincident with the lowered arterial pressure during expiration (down stroke of respiration lever) has flattened troughs (diastole), while with the higher arterial pressure during inspiration there is no flattening of the troughs. In the first case the artery has collapsed during diastole, in the second case it has remained patent for the whole cardiac cycle. Contrasting this region of the record with that at 120 mm. Hg. external pressure and that at 71 mm. Hg. external pressure it is seen that in the former the artery collapses with each and every diastole, while in the latter the record indicates that it is patent during the whole cardiac cycle and we infer (since a sound is produced) that it suffers only a certain degree of flattening. It was observed in our experiments indeed that the change from the phase of collapse to the phase in which collapse does not occur is marked by a change in the character of the sounds heard. A schematic representation of events as they occur in this record together with their relation to sound production has been prepared in figure 2. The explanatory note describes it in detail.

On the basis of the water hammer theory of sound production (Erlanger) one finds an explanation of the changing character of the sounds (as external pressure is lowered) which is most suggestive and adds something to our notion of their significance and value as criteria. As external pressures are lowered below the systolic level, the intensity of the sound increases to a certain maximum of intensity and then *suddenly* diminishes. From this point on the sounds become fainter and finally disappear. One should be able to explain the changing intensity by a corresponding change in water-hammer-pressure. The evi-

dence of changing water-hammer pressure can be found in the character of the pulse wave (length of obliteration period), while the changes in sound intensity may be observed and the relation of the one to the other noted.

If during the cardiac cycle the artery underneath the cuff becomes obliterated, the column of blood distal to the cuff will become practically motionless (being moved only by contraction of the walls of the distal segment), since no blood can pass the obliterated segment and enter it from above. Moreover the degree to which this distal column of blood is slowed cannot be greatly influenced by the length of time during which obliteration lasts. Once arrested by obliteration, continued obliteration can add little to the effect. Under these circumstances one factor in the production of a water hammer pressure is at its maximum. On the other hand, the obliterated segment offers a resistance to the pulse wave proceeding through the proximal segment and serves to reduce its force and velocity before its impact upon the distal segment occurs. Though the length of the obliteration period does not materially effect the velocity of the distal column, yet as the period is shortened there will be a corresponding increase in the force and velocity with which the proximal column strikes the distal column (compare Erlanger (5), p. 86). Therefore as the obliteration period is shortened with the lower external pressure there is an increase of water hammer pressure with each lowering of external pressure. This increase should continue to the point at which obliteration fails to occur. When the external pressure is lowered to a point at which no obliteration occurs, the distal column of blood is kept moving throughout diastole, for blood is allowed to pass the segment underneath the cuff and enter the distal artery. The impact of the proximal column upon this moving distal column will result in a decreased water hammer pressure being produced. The transition from a stagnant to a moving distal column is sudden, and there is a correspondingly sudden decrease in water hammer pressure. Also we should expect at this point a sudden decrease in the intensity of sound. The relation of the obliteration period and its effect on the factors producing a water hammer pressure as we conceive it, is shown in the diagram (fig. 3).

Evidence of this sequence of events together with its relation to sound production is obtained from the record in figure 1. Changes in the water hammer pressure may be followed by the reader, as it was observed in our experiments, by the changing length of time during which the trough of the pulse wave runs horizontally. The horizontals

(in trough of pulse wave) indicating obliteration decrease in length as the sound becomes more intense to the observer. The disappearance of these horizontals indicating no obliteration was attended by the sudden change in the intensity of the sound. Obviously the external pressure sufficient to cause obliteration is somewhat above the lowest (diastolic) pressure occurring in the artery during a cardiac cycle,

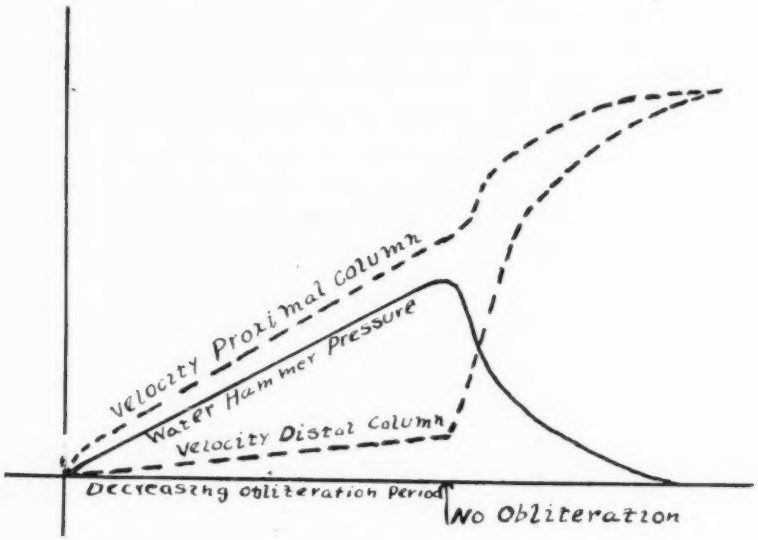


Figure 3

hence the change of sounds is not to be taken as an index of diastolic pressure. From this point down sounds continue. A point is finally reached where no sound is produced. We are led to believe by inference that this lower period of sound production is attended by some deformation of the arterial wall. The level of external pressure at which no deformation occurs should be equal to the diastolic pressure. At this level the internal and external pressures simply equalize each other.

THE QUANTITATIVE DETERMINATION OF THE RESPIRATORY CHANGE OF ARTERIAL PRESSURE

As stated in an earlier section the Korotkoff sounds may also be used as an index in the measurement of the extent of blood pressure change accompanying respirations.

During a decompression experiment the level at which the first few sounds are heard (or at which the period of sounds is first introduced) must be the level of the highest systolic pressure during a respiratory cycle. This may be called the *maximum respiratory systolic pressure.*

When upon further decompression the sounds are constant for the first time throughout the whole of the respiratory cycle, the level of the lowest systolic pressure is reached. This pressure may be called the *minimum respiratory systolic pressure.*

Similarly when further decompression brings one into the diastolic region, there will be a transition from constant sounds during the whole of respiration to a level where the sounds begin to take on a periodic character again. The pressure at which this second periodicity of sounds just begins is taken as the highest diastolic level and has been called the *maximum respiratory diastolic pressure.*

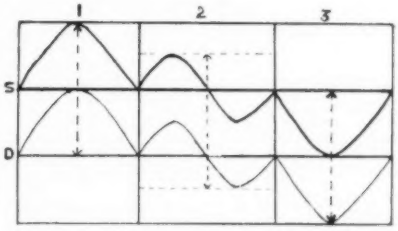


Figure 4

Upon still further decompression even the periodic sounds drop out. The level of pressure at which the period of silence for the first time covers the whole of a respiratory cycle is taken as the lowest diastolic pressure during a respiration and has been called the *minimum respiratory diastolic pressure.*

It should be stated at this point that the directions in which deep respirations influence the maximum and minimum systolic and diastolic levels away from what would be (mean) systolic and diastolic pressures with quiet breathing may be various. This is graphically shown in figure 4, where three cases are plotted.

(1) The maximum systolic is raised above the systolic level of quiet breathing, while the systolic level of quiet breathing now becomes the minimum systolic.

(2) In addition to the raising of the maximum systolic above the

level of normal breathing, there is a fall of the minimum systolic below this level.

(3) The systolic level of normal breathing becomes the maximum systolic while the minimum is reduced to a point below the systolic level of normal breathing.

The nature of the change in the diastolic level is the same as that in the systolic level. These combinations are put to schemata in the figure 4. Probably in the usual case something resembling (2) is what actually occurs—a combination of (1) and (3).

In any case the "Respiration pulse pressure" (heavy broken lines) far exceeds the ordinary pulse pressure (distance from S to D, the systolic and diastolic levels of quiet breathing).

METHOD AND RESULTS

The usual wide-cuff compression bag was applied to the arm enclosing the brachial artery. In the path of the compression chamber were inserted the usual mercury manometer, inflation bulb, needle valve and Marey sphygmoscope. The latter connected directly with a double lever Marey tambour. Instead of the parts of the apparatus being crowded together compactly as is usual in a sphygmomanometer for bedside use, they were strung out in a linear arrangement with the sphygmomanometer lever at one end free to be applied to the writing surface of an ordinary drum kymagraphion.

Provision was made for simultaneous tracings on the drum record for the following:

(a) The blood-pressure, or sphygmomanometer lever.

(b) The mechanical respiratory movements. A lever with Ludwig writing tip (chord recording lever) traced these movements. The transmission of the movement was done by a pneumatic system of elastic bags, with a Marey bulb intercalated.

(c) An electro-magnet signal lever to record sounds. The key of this lever was operated in the hand of the person listening for the Korotkoff sounds. Every sound was thus recorded throughout an experiment. The loss of time (mechanical and reaction time of the observer) in recording the separate sounds was neglible in this work because we were not trying to determine at what point of time in the cardiac cycle the sound was produced. The method of stopping the drum at each change of level of cuff pressure automatically[3] marked off on the record the portion belonging to each of the pressure levels.

[3] By means of the scratch marks of the moving lever tips. Thus also does one have a continuous control of the vertical relation of the writing tips.

In case continuous decompression was elected

(d) a fourth signal lever operated by a key in the hand of another observer marked off each 5 mm. fall in manometer pressure. This was only employed in continuous decompression experiments. In most of the experiments the subjects were required to breathe as deep and slowly as they could with comfort for the duration of an experiment.

A type of the graphic records thus obtained appears in figure 1, scrutiny of which will readily show:

(a) The periodicity of the Korotkoff sounds in the regions of systolic and diastolic pressures.

(b) The inversion, or reversal, of the position of the "period of sounds" in relation to the "period of silence" of a respiratory cycle in the systolic region when compared with a respiratory cycle in the diastolic region.

(c) The inversion of the respiratory wave in the sphygmomanometer trace as described in a previous communication.

(d) The levels at which maximum respiratory systolic pressure occurs—shortest period of sounds, and minimum respiratory systolic pressure—shortest period of silence, or transition from periodic to constant sounds, both in the systolic region of the sphygmomanometer trace.

(e) The levels at which the maximum and minimum respiratory diastolic pressures occur—maximum and minimum periods of sounds in the region of diastolic pressure.

(f) The inspiratory rise and expiratory fall of blood pressure is shown by position of the "period of sounds" in relation to the phases of the respiration. See trace of respiratory movements. Upstroke of lever is the inspiratory movement.

(g) The close correspondence between the periodicity of the sounds and the rise and fall of the cardio-respiratory wave in the sphygmomanometer trace.

(h) The relation of changing heart rate to rise and fall of blood pressure.

Most of the experiments were intermittent decompression experiments, the pressure intervals being 5 mm. The order of events therefore is the order as they occur with cuff-pressure passing from higher to lower manometer levels. And this also is, as far as the data are concerned, the order adopted in recording the results as shown in table 1.

Stress for a long time has been laid upon the importance of pulse pressure in the normal experimental perfusion of the various organs of the

TABLE 1

Deep breathing experiments

NUMBER OF EXPERIMENT		RESPIRATORY SYSTOLIC PRESSURES		RESPIRATORY DIASTOLIC PRESSURES		RESPIRATORY PRESSURE RANGES	
		Maximum	Minimum	Maximum	Minimum	Systolic	Diastolic
(1)		(2)	(3)	(4)	(5)	(6)	(7)
I	1	133	103	68	55	30	13
	2	138	103	50	30	35	20
	3	145	110	95	60	35	35
	4	130	110	76	62	20	14
	5	125	115	68	55	10	13
	6	140	112	90	74	28	16
	7	125	104	66	45	21	21
II	8	125	80	50	35	45	15
	9	140	100	60	50	40	10
	10	130	110	70	55	20	15
	11	115	90	60	36	25	24
	12	126	90	60	36	36	24
	13	130	85	60	40	45	20
	14	135	110	62	50	20	8
III	15	134	105	65	40	29	25
	16	140	120	72	50	20	22
IV	17	100	80	65	50	20	15
V	18	130	95	81	78	35	3
	19	128	106	95	76	22	19
VI	20	133	113	100	85	20	15
VII	21	125	98	60	50	27	10

Explanatory note to Table 1

Column 1. The number of experiment is merely here indicated. Data concerning the conditions of the experiment will be found in Table 2 after the corresponding numbers. The Roman numerals refer to different subjects.

Column 2. The level of cuff-pressure at the moment when the periodic sounds first were heard are here listed. The level is styled the *maximum respiratory systolic pressure.*

Column 3. The level of cuff pressure at the moment when the sounds lose their periodicity with reference to the respiration is noted in this column. The pressure is styled the *minimum respiratory systolic pressure.*

Column 4. The level of cuff-pressure at the moment when, during further decompression the sounds again first fail to be heard for a portion of the respiration interval. This pressure is called the *maximum respiratory diastolic.*

Column 5. The level of cuff-pressure when the period of sounds becomes reduced to one or two pulse beats per respiration, or the level at which for the first time no sounds are heard at all during a respiration. Styled, *minimum respiratory diastolic pressure.*

From the observed data contained in these four columns may now be calculated the following:

Column 6. The difference between maximum and minimum respiratory systolic pressures. This gives the *respiratory systolic range.*

Column 7. The difference between the maximum and minimum respiratory diastolic give the *respiratory diastolic range.*

body, in physical examinations, and in clinical diagnosis of pathologic conditions (2). Pulse pressure is indicative of a combination of well known factors, such as elasticity of arterial bed, rate and force of heart-beat, etc.

The act of respiration may throw into the whole circulatory system another oscillation wave of equally great magnitude, synchronous not with the heart-beat, but with the respiration itself. Upon this wave the more frequent oscillations of the heart-beat are superimposed. To condense the picture of the cardiac effect in a single term the earlier authors chose the expression, pulse-pressure. To condense the picture of the respiratory effect in a single term one may similarly speak of the *respiratory-pressure*. This would be the difference between the two systolic levels or diastolic levels, or the effect as expressed by the terms (see columns 6 and 7 of table 1) respiratory systolic or respiratory diastolic range.

The combined effect of these two great oscillatory changes, pulse pressure and respiration pressure, with all their underlying physiological factors may likewise, therefore, be summed up in the term, *Respiration-Pulse-Pressure*.

The data for the calculation of this pressure will likewise be found in table 1. From the maximum respiratory systolic one simply subtracts the minimum respiratory diastolic pressure. This has been taken as the measure of respiration-pulse-pressure, for obviously the "maximum respiratory systolic" would not have been so high nor probably the "minimum respiratory diastolic" so low, but for the act of deep respiration.

The respiration-pulse-pressure as thus measured from our data are shown in table 2. Here also are added other data belonging to the experiments which for convenience of printing do not appear in table 1. The mean pulse pressure for each case is also added for comparison. The data for the mean pulse pressure will be found in table 3.

Determinations of the mean pulse pressure (and of both systolic and diastolic pressures) should have been made while the subjects were breathing shallow or holding the breath in each experiment for controls. This will be done in the work to follow. The increase in pressure changes of the deeper breathing compared with the normal breathing may be even more striking than the increase shown in table 2. Be that as it may, if one takes the ratios of the increase of respiration-pulse-pressure over and above that of the corresponding mean pulse pressure for the deep breathing experiments as shown in table 2, one

TABLE 2*

NUMBER OF EXPERIMENT		SUBJECT	DATE	CHARACTER OF BREATHING	RESPIRATION PULSE PRESSURE	ORDINARY PULSE PRESSURE
I	1	R. C.	11/5/15	Abdominal-thoracic	78	57
	2	R. C.		Abdominal-thoracic	108	80
	3	R. C.	18/5/15	Abdominal-thoracic	85	50
	4	R. C.	3/12/15	Abdominal	68	51
	5	R. C.	3/12/15	Thoracic	70	59
	6	R. C.	15/12/15	Abdominal	66	44
	7	R. C.	26/2/16	Thoracic	80	59
II	8	F. F.	4/5/15	Abdominal-thoracic	90	60
	9	F. F.	18/5/15	Abdominal-thoracic	90	65
	10	F. F.	18/5/15	Abdominal-thoracic	85	58
	11	F. F.	1/12/15	Thoracic	79	54
	12	F. F.	1/12/15	Abdominal	90	60
	13	F. F.	1/12/15	Abdominal	90	57
	14	F. F.	26/2/16	Thoracic	85	68
III	15	J. G. H.	18/5/15	Abdominal-thoracic	94	67
	16	J. G. H.	18/5/15	Abdominal-thoracic	90	69
IV	17	L. K.	1/12/15	Thoracic	50	33
V	18	C. S.	4/12/15	Thoracic	52	34
	19	C. S.	4/12/15	Abdominal	52	42
VI	20	J. H.	18/8/15	Abdominal-thoracic	48	31

* See tables 1 and 3 for the data from which these pressures are determined.

TABLE

Mean arterial pressures in deep-breathing experiments in mm. of mercury

NUMBER OF EXPERIMENT		SYSTOLIC	DIASTOLIC	PULSE PRESSURE*
I	1	118	61	57
	2	120	40	80
	3	127	77	50
	4	120	69	51
	5	120	61	59
	6	126	82	44
	7	114	55	59
II	8	102	42	60
	9	120	55	65
	10	120	62	58
	11	102	42	54
	12	108	48	60
	13	107	50	57
	14	122	54	68
III	15	120	53	67
	16	130	61	69

* This pressure is obtained from the difference of the mean systolic and mean diastolic. Practically, though not invariably, the same figures are obtained by taking the difference between maximum systolic and maximum diastolic or between minimum systolic and minimum diastolic pressures.

finds the increase to vary from 19 per cent to 58 per cent. The average of all the percentages is very nearly 40 per cent.

GENERAL REMARKS

Since the magnitude of respiratory pressure varies with the algebraic sum of the respiration factors it at first did not appear that there could be any constancy in the numerical values of this pressure. Refinement of analysis and method may alter the absolute values of our figures. In any event we must bear in mind that the respiration pressures are a function of depth and time interval of the respiratory act and may, and do, vary greatly *in* the individual as well as *among* individuals, as has been known all along and as our experiments show. The big gap in our knowledge has been *how much* does blood pressure vary with respiration, and *how much can it be made to vary*. Our figures answer these questions more definitely than heretofore has been attempted.

It should be stated here again that during our experiments, while the subjects were asked to breathe deeply and slowly, they were asked to do so only to the extent to which they could still feel comfortable. Had they breathed to the fullest capacity of their lungs the values of respiration-pulse-pressure would doubtless have been still greater than these we have recorded.

In any case our experiments show beyond a doubt that during deep breathing there is an ebb and flow of the blood throughout the tissues on a much greater scale than physiologists probably would have supposed.

When one looks at the unexpected magnitude of respiration-pulse-pressure one is inclined to question the reckless recommendation to the innocent public of deep-breathing as a harmless form of physical exercise.

Just as pulse pressure has been of so much significance to the physiologist, physical director and clinician, it is believed that the determination of respiration-pulse-pressure, when once sufficient data are gathered, may prove to be of even more far reaching service in the estimation of physical fitness, in the determination of the character of and damages done by disease, and possibly in the more intelligent prescription of physical exercise.

SUMMARY

1. A method for the determination of the character of respiratory change of arterial pressure in man by means of the Korotkoff sounds is described.

2. An extension of this method is further described whereby one may determine the extent of arterial pressure changes in man accompanying respirations. The pressure change so determined may be expressed in terms of mm. of Hg.

3. The difference between the maximum systolic and minimum diastolic pressures occurring during a respiration has been called the *respiration-pulse-pressure.*

4. In deep breathing experiments it is shown that the respiration-pulse-pressure may be from 19 to 58 per cent greater than the ordinary pulse pressure.

5. A discussion of the significance of the Korotkoff sounds and of their relation to certain characteristics in the corresponding sphygmomanometer tracing, and to the critical arterial pressures in man, is included in the body of the paper.

6. It is shown that the extent and duration of the obliteration of the artery in a decompression experiment may be seen in the sphygmomanometer tracing by the flattening of the troughs of the pulse waves.

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THE CONDUCTION OF PAINFUL AFFERENT IMPULSES IN THE SPINAL NERVES

Studies in Vasomotor Reflex Arcs. II[1]

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Within the skin are endorgans capable of responding to tactile, thermal and painful stimuli. Impulses arising in such endorgans are propagated along afferent nerve fibers to the central nervous system. Within the spinal cord the tactile, thermal and painful afferent impulses follow separate paths toward the brain—paths which have been mapped with considerable accuracy. But we have not known whether in a peripheral nerve a single fiber could convey only one, or several kinds of afferent impulses. If it be assumed that a specific set of fibers mediates each variety of cutaneous sensation, how may we differentiate, for example, those mediating pain from the other afferent fibers? This entire question has been very obscure because of the absence of any adequate knowledge as to the structure and function of the afferent fibers in the peripheral nerves. Only recently has our information along these lines become sufficiently precise to make possible a solution of the problem.

We have attacked the problem along lines suggested by these recent observations and have conducted a series of experiments the results of which are very convincing. It will be necessary, before giving an account of the experiments, to summarize the recent anatomical and physiological observations, a consideration of which led us to make the experiments.

[1] The first paper of this series was published under the title "The conduction within the spinal cord of the afferent impulses producing pain and the vasomotor reflexes," this Journal, xxxviii, p. 128.

STRUCTURE OF THE AFFERENT CEREBROSPINAL NERVE FIBERS

During the last six years (1) it has been shown by means of a new differential axon stain that the spinal nerves contain more unmyelinated than myelinated fibers. These numerous unmyelinated fibers had not been seen before because they could not be stained by any of the methods previously used. They have been demonstrated in great numbers in the vagus as well as in the spinal nerves and are probably present in other cranial nerves also. It has been shown that these fibers in the spinal nerves arise from the small cells of the spinal ganglia. These, like the larger cells of the ganglia, are unipolar with a process that divides dichotomously. One branch runs peripherally along the spinal nerve, the other runs centrally along the dorsal roots to the spinal cord. Both remain unmyelinated throughout their course. That these small cells of the spinal ganglion and the associated unmyelinated fibers are afferent elements is shown by the location of the cell body in the spinal ganglion and its conformity to the typical structural type of afferent neurones.

Most of the unmyelinated fibers in the spinal nerves go to the skin; a few go to the deeper structures. Traced centrally along the dorsal roots, they are seen to run into the tract of Lissauer of the spinal cord. As the root approaches the spinal cord it breaks up into a number of fine radicles which spread out in a longitudinal direction and enter the cord along the posterolateral sulcus. Within each radicle as it approaches this sulcus the unmyelinated separate out from among the myelinated fibers and take up a position around the circumference of the radicle and along septa that divide it into smaller bundles. Then, as indicated in figure 1, these unmyelinated fibers run toward the lateral side of the radicle and, leaving it just as it enters the cord, they turn ventrolaterally into the tract of Lissauer. They are accompanied by a few fine myelinated fibers; but almost all of the myelinated fibers run medialward over the substantia gelatinosa into the fasciculus cuneatus. This fasciculus receives practically none of the unmyelinated fibers. We shall speak of the bundle of unmyelinated fibers that turns ventrolaterally into the tract of Lissauer as the lateral division of the root, and of the myelinated fibers that run over the substantia gelatinosa into the cuneate fasciculus as the medial division. The fibers of the lateral division are extremely fine and closely packed together, so that it is small as compared to the medial division, which consists of very much coarser fibers. Yet in spite of its small size, the lateral

contains fully as many if not more fibers than the medial division, as can be readily understood when one considers the great difference in the size of the contained fibers. The number of the unmyelinated fibers could not be adequately represented in a low power drawing like that represented in figure 1.

The unmyelinated fibers run up or down in Lissauer's tract for only a very short distance, usually less than a segment. They then turn into and end in the substantia gelatinosa, which is to be regarded as the nucleus of reception of these fibers. That is to say, these fibers run into the gray matter at or near the level at which they enter the cord. Their intraspinal course suggests at once that they are the fibers of pain and temperature sensations, since it is known that the afferent impulses underlying these sensations pass through the gray matter as soon as they reach the cord. This brings us to a consideration of Head's important work on pain and temperature sensations.

PROTOPATHIC NERVE FIBERS

An important advance was made by Head and his associates (2) when they showed that cutaneous sensations could be separated into two groups, to which they applied the terms protopathic and epicritic. Under the term protopathic Head groups pain and the temperature sensations aroused by objects under 22° or over 40°C. This group is characterized by a "peculiar tingling quality," by radiation into other parts than those stimulated, and by failure of the subject to localize accurately the point stimulated. Under the term epicritic he groups sensibility to light touch, temperature sensations derived from objects between 22° and 40°C., and discrimination of the two compass points. Sensations of this sort are all accurately localized. It would take too much space to tell in detail how these two types of sensation were separated from each other. Obviously such a distinction could not be made by a study of the normal skin. It was found, however, that after lesions of the dorsal roots areas of pure epicritic sensation appeared. Such a cutaneous area was sensitive to light touch and to medium degrees of temperature, but insensitive to pain and to the more extreme degrees of temperature. On the other hand, when the median nerve was cut a cutaneous field became outlined on the palm of the hand, in which only protopathic sensations were experienced. Here the skin was sensitive to pain and to the extreme degrees of temperature, but insensitive to light touch and the intermediate degrees of temperature.

All sensations from such an area were poorly localized and had a peculiar tingling quality. Head and his associates studied a very large series of cases with nerve lesions and found many such areas of dissociated sensation.

It is maintained by Head that each of his two sensory groups depends on a separate anatomically distinct set of nerve fibers. He presents good and convincing reasons for this belief, but space does not permit us to repeat the argument here. We wish only to call attention to a fact which might easily be overlooked in reading the original articles. In areas of partial anaesthesia the residual sensation may be either protopathic or epicritic. If the only form of residual sensation were protopathic one might assume that it depended only on a decreased density of innervation. It might easily be that light touch required a denser innervation for its perception than pain. But the reverse form of partial anaesthesia also occurs (and numerous examples of it are given by Head) in which epicritic sensation persists over an area devoid of protopathic sensation. It is not conceivable that a simple decreased density of innervation should in the one case give rise to a loss of light touch with pain persisting and in another case cause a complete loss of pain sense while light touch remains normal. Furthermore, these areas of pure epicritic sensation are sensitive to temperature between 22° and 40°, but insensitive to the more extreme degrees of temperature. This is clearly not a case of lowered sensibility due to decreased density of innervation. It seems clear to us that these facts can be explained only on Head's assumption that there are two kinds of afferent nerve fibers, which differ slightly in their anatomical distribution.

According to Head, the unit of distribution of protopathic fibers is the dorsal root, each root having a sharply outlined area of skin which it supplies with them. The epicritic fibers of adjacent roots are intermingled in their cutaneous distribution. Section of one or more dorsal roots deprives a sharply circumscribed area of skin of its protopathic fibers, while epicritic fibers from adjacent roots run into this area, endowing more or less of the skin near its border with pure epicritic sensation. Here light touch is felt, but not pain, warm and cold objects are discriminated but hot and cold objects give rise to no temperature sensation. In the same way the peripheral nerve is the unit of epicritic sensation. The epicritic fibers of the ulnar nerve are limited to the area of skin outlined by anatomists as representing the cutaneous distribution of that nerve; but the protopathic fibers of the ulnar run

long distances in the subcutaneous plexuses into the areas belonging to adjacent nerves. When the median nerve is cut epicritic sensation is lost over the entire area ordinarily assigned by anatomists to that nerve, but protopathic fibers from the ulnar nerve run into this area endowing a considerable extent of the skin near the border of the area with pure protopathic sensation. Here pain is felt but not touch. Hot and cold objects are distinguished, but warm and cool objects give rise to no temperature sensation.

Facts which are otherwise inexplicable are thus readily understood on the assumption of two kinds of nerve fibers which vary slightly in their anatomical distribution. This assumption acquires still greater significance in view of the recent demonstration that there are two kinds of afferent cerebrospinal nerve fibers which differ both in structure and distribution.

As we have seen in the first section of this paper, there are in the cerebrospinal nerves, great numbers of unmyelinated fibers which had been previously overlooked. The most striking parallel exists between what is known of the protopathic fibers and what has recently been determined in regard to the unmyelinated fibers. This comparison can be carried out with great detail and with the most convincing results; but it involves many details which have no place in this paper and have been presented elsewhere (3). The course of the afferent fibers in the dorsal root and spinal cord, has, however, a direct bearing on the present investigation and must be considered here.

It is well known that the afferent impulses underlying sensations of pain and temperature must pass through the gray matter and cross to the opposite side of the cord at or near the level at which they reach it. Head (2) has shown that this is true for temperature sensation of the epicritic as well as of the protopathic order. According to him, the other elements of the epicritic group (touch, tactile discrimination and tactile localization), are carried upward on the same side of the cord in the posterior funiculus for varying distances before ending in the gray matter. He maintains that the tactile impulses coming in along a given root do not cross to the opposite side of the cord all at once, but that they ascend in the posterior columns for varying distances. The crossing at various levels, of impulses coming in by a single root gives rise to a double pathway for touch, uncrossed fibers of the first order paralleling crossed fibers of the second order for a certain number of segments. This double path no doubt accounts for the conflicting observations on the conduction of tactile impulses, which are found in the literature.

The facts that have been ascertained regarding the intraspinal course of the unmyelinated fibers are in complete accord with the view that they are the conductors of protopathic afferent impulses. As a dorsal root enters the spinal cord the two kinds of fibers separate; the unmyelinated turn laterally into Lissauer's tract, while the myelinated run on into the posterior funiculus. Few, if any, unmyelinated fibers enter that funiculus, but a few fine myelinated fibers run into the tract of Lissauer. This consists chiefly of unmyelinated axons, scattered among which are a few fine myelinated fibers. From the level at which they enter the cord these fibers ascend or descend in the tract for a very short distance not exceeding one or two segments. The substantia gelatinosa seems to be the sensory nucleus associated with this tract.

The unmyelinated fibers, then, enter the gray matter at or near the level at which they enter the cord. In this they are in exact agreement with the fibers conveying protopathic sensation. The myelinated fibers, which alone enter the posterior funiculus, correspond in their intramedullary course to the fibers carrying light touch, tactile discrimination and tactile localization, since according to Head these ascend for longer or shorter distances in this funiculus before entering the gray matter. As to the temperature sensations in the epicritic range, they are probably conveyed by the fine myelinated dorsal root fibers that run with the unmyelinated ones into the tract of Lissauer. It is thus apparent that we have at hand data sufficient to explain the intramedullary course of the protopathic and epicritic sensations in terms of the demonstrated intramedullary course of the myelinated and unmyelinated fibers.

The function of the tract of Lissauer. Experiments on the spinal cord of the cat (4) have shown that the tract of Lissauer and the substantial gelatinosa Rolandi are at least closely associated with the pain reception and conduction apparatus. It was found that while bilateral destruction of the tract of Lissauer and the substantia gelatinosa at the level of the first lumbar segment of the cat's cord did not interfere in any way with the perception of pain in the hind limb, it entirely eliminated the pressor vasomotor reflex from stimulation of the sciatic nerve. Now, the vasomotor reflexes are distinctly protopathic in that they are produced almost exclusively by pain and temperature sensations. The evidence presented in that paper showed that the tract of Lissauer and the substantia gelatinosa formed a path for the conduction of the afferent impulses involved in the reflex vasoconstriction due to painful sciatic stimulation. It seemed probable to us that the tract

of Lissauer and the substantia gelatinosa Rolandi formed an apparatus for the reception and intersegmental conduction of painful afferent impulses. Some impulses from this apparatus passing over to the spinotholamic tract would reach the cortex and find expression as conscious pain, while other impulses received in this apparatus would ascend and descend within it, producing pain reflexes. So far as the evidence goes, this work favors the theory that the unmyelinated fibers conduct protopathic sensation, in that it shows that the portion of the cord in which these fibers run and terminate forms part of a protopathic reflex arc.

STATEMENT OF THE PROBLEM AND OUTLINE OF THE EXPERIMENT

It occurred to us that the sharp separation of dorsal root fibers to form the medial and lateral divisions of the root could be made the basis of some interesting experiments. By raising the root and cutting in the direction of the arrow *A* in figure 1, the lateral could be cut without injuring the medial division. On the other hand, by a cut in the direction of line *B* in figure 1 the medial could be cut without injuring the lateral division. Stimulation of a root before and after such an operation might yield information concerning the sort of afferent impulses that enter the cord by way of each of these two divisions of the root, and concerning the function of the myelinated and unmyelinated fibers.

TECHNIQUE

Adult cats were used for the experiment. The spinal canal was opened by removal of the spinous processes and laminae from the fifth lumbar to the first sacral vertebra inclusive. In all but the first three experiments this was done as a preliminary operation under rigid asepsis and the animals were allowed to recover from the loss of blood and shock of this operation for a period of five to ten days. The three cats in which the exposure and experiment were carried out under one anaesthetic had a very low blood pressure, but those which were allowed to recover from the preliminary operation showed a normal blood pressure during the subsequent experiment.

During the experiment the animals were under ether anaesthesia. A tracheotomy was performed and an ether bottle attached. Care was taken to maintain a constant and rather light grade of anaesthesia. Connections were made to secure carotid blood pressure tracings. The animal was then placed on an animal board so arranged that the weight

was borne entirely by the upper part of the thorax and the pelvis—the lower part of the thorax and the abdomen being free to move during respiration without coming in contact with the board.

The dura mater was opened by a median dorsal incision corresponding to the length of the defect in the bony canal. The last large root was selected for the experiment, and at the autopsy this was found

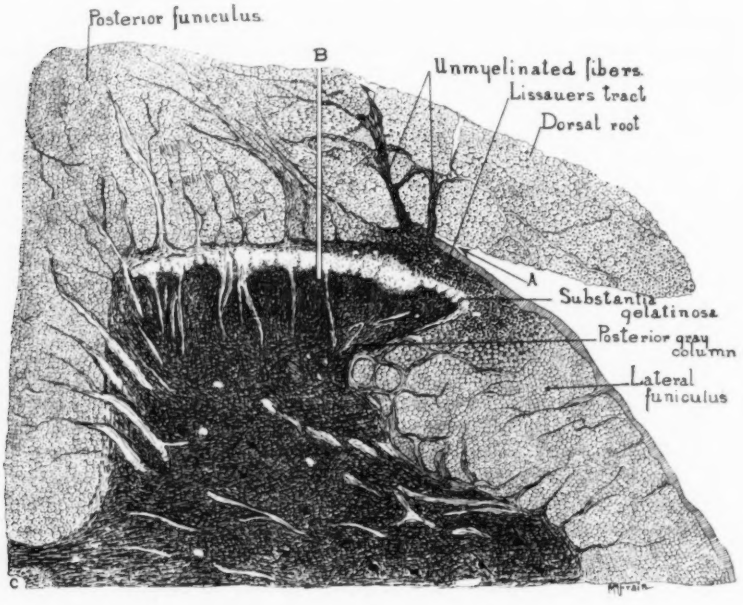


FIG. 1. From a section of the seventh lumbar segment of the spinal cord of the cat. Arrow *A* indicates the direction of a cut through the lateral division of the root. Line *B* indicates the direction and extent of a cut through the medial division of the root.

to have been the last lumbar in every case except two in which it was the first sacral. A ligature was passed around the root selected at the level of the ganglion; the ligature was tied and the nerve cut distal to it. The nerve was raised by traction on the ligating thread and the ventral root was divided just proximal to the ligature. The preparation then consisted essentially of a dorsal root ligated and cut distal to the ligature, but still attached centrally to the spinal cord. The sev-

enth lumbar and first sacral roots are long and furnished a preparation 2 or 3 cm. in length. They were chosen partly because of their length and partly because the separation into medial and lateral divisions is very evident in these roots. The two divisions are so placed as to be readily divided separately.

During stimulation the root was elevated by gentle traction on the ligating thread. Standard platinum electrodes were applied a short distance proximal to the lagature. The stimulus was a faradic current derived from a Stoelting inductorium No. 7090, through the primary of which was passed a constant half ampere current. The position of the secondary varied from 5 to 8. Note was made of the changes in respiration and any struggling produced by the stimulus and the vasomotor reflexes were recorded on the kymograph.

After a record had been taken of the results of stimulating the root, the desired cut was made with a sharp iridectomy knife. The knife was drawn carefully along either the medial or the lateral side of the entering root. The incisions along the lateral side of the root in the direction of the arrow in figure 1 were always very restricted in extent. The cuts on the medial side varied, in two cases extending deeply into the cord as indicated by the line *B* in figure 1. After the cut had been made the root was stimulated again in the same manner and with the same strength of current as before.

The rootlets into which each root divides as it enters the cord are very small and the preparation requires careful handling. On account of the small size of the rootlets it was felt that there was danger that they might become chilled or dry during the experiment. In order to prevent such an error the cord and roots were kept flooded with normal salt solution at 39°C., except during the time of stimulation. Preceding each stimulation the saline was removed with absorbent cotton and the cut end of the root elevated by gentle traction on the ligating thread. Since the preparation was at least 2 cm. long and the stimulus was applied close to the ligature, there was no danger of an escape of current to the cord or other roots.

After one root had been tested in this way the corresponding root of the opposite side was used. In most of the cats the medial division of one root was cut and the lateral division of the other. In this way it was possible to compare the effects of the two lesions in the same cat under the same conditions of anaesthesia, blood pressure and vasomotor irritability.

The animal was then autopsied and the roots identified. They were

usually the seventh lumbar, but twice the first sacral. The roots and corresponding segment of the cord were removed together and cut into serial sections stained by the pyridine silver technique. These sections were studied under the microscope and the exact amount and character of the damage done in each case was determined.

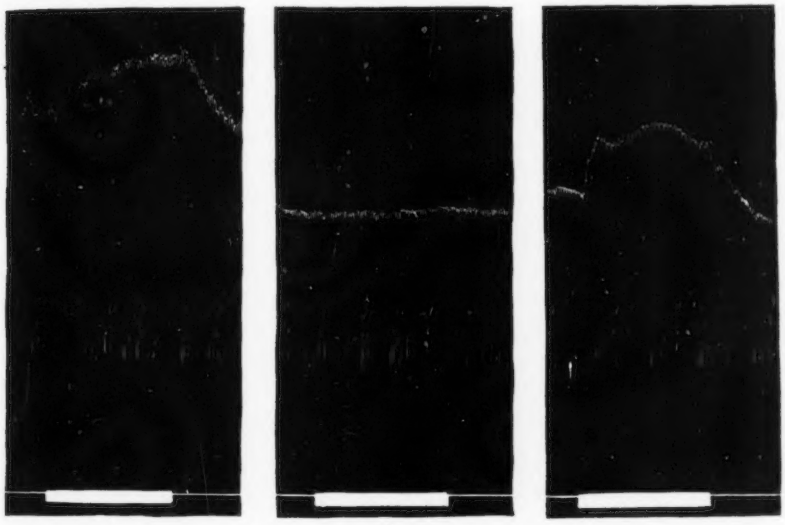
SECTION OF THE LATERAL DIVISION OF THE DORSAL ROOT

The lateral division of the root was cut in three first sacral nerves—on one side in one cat and on both sides in another cat—and in three seventh lumbar nerves—one nerve in each of three cats. In two of these six experiments stimulation of the root preceding the section of the lateral division gave rise to some struggling, although the animals were under moderately deep anaesthesia. This struggling was not elicited by stimulation of the same root with the same strength of current after the lateral division had been cut, although it could still be elicited by stimulating other nerve roots. Stimulation of the intact root resulted in an increase in rate and depth of respiration; the same stimulation after section of the lateral division gave rise to no change in respiration. In each of the six experiments stimulation of the intact root caused an increase in blood pressure—the typical pressor curve—and in each instance the pressor reflex was found to have disappeared after section of the lateral division of the root. In most cases these results were checked by a subsequent stimulation of another root which gave both the pressor reflex and changes in respiration showing that the respiratory and vasomotor centers were still functioning normally. It is evident that the lesion, which was a very superficial one on the lateral aspect of the entering root, prevented the entrance into the cord of those afferent impulses that cause struggling, increased rate and depth of respiration, and the pressor vasomotor reflex. In four experiments the pressor reflex was entirely obliterated and in the other two there remained only the slightest trace of this reflex.

In each case the cord segment and dorsal root involved were stained by the pyridine silver method and cut into serial sections which were carefully studied under the microscope. These showed that the lesion had been accurately placed and was very restricted. Although a majority of the fibers of the lateral division had been cut, some had escaped. In only one experiment was the division of this part of the root complete; but in every case it had suffered serious damage. In none of these experiments had the medial division of the root been injured.

The medullated fibers of the root could be followed into the cuneate fasciculus and showed no evic ce of having been damaged by the laterally placed cut.

As an illustrarion of the results obtained we will cite the details of one experiment:



a b c

FIG. 2. Carotid blood pressure tracing from Cat 64. *a*, Strong faradic stimulation of the left seventh lumbar dorsal root; *b*, same stimulation of the root after its lateral division had been severed; *c*, same stimulation of the right seventh lumbar root after a cut had been made on its medial side as extensive as the cut on the lateral side of the left root.

Cat. 64, adult. Preliminary operation of opening the spinal canal December 3, 1915. Experiment December 14, 1915. Ether anaesthesia. Wound in skin and muscles opened and dura exposed. Tracheotomy. Ether bottle. Carotid canula. Dura opened. Cord and roots kept flooded with warm normal salt solution except during stimulation. Ligature passed around the left seventh lumbar nerve, tied, and the nerve cut distally. Ventral root cut near the ligature. Dorsal root gently raised by the ligating thread and electrodes applied close to the ligature. Faradic stimulation fifteen seconds with the secondary coil at 5. Result: Good pressor reflex—figure 2a; increased rate and depth of respiration, the increased rate being indicated in figure 2a by the obliteration of the respiratory wave in the blood pressure tracing; some struggling. A very small cut along the lateral side of the root in the direction of the arrow in figure 1. Root

stimulated as before. Result: No struggling; no change in respiration; no change in blood pressure—figure 2b. A study of serial sections shows that the lateral division of the root was completely cut with practically no injury to the medial division.

SECTION OF THE MEDIAL DIVISION OF THE DORSAL ROOT

To the preceding experiments the objections might well be raised that the rootlets, emerging in linear order from the posterolateral sulcus and uniting to form the dorsal root, are so very fine that it would be impossible to make the cut described without traumatizing them to such an extent as to render all the fibers nonconductive. In order to meet this objection it was necessary only to subject the medial side of the root to the same amount of trauma. Now, the medial division is much larger than the lateral and a cut which was sufficient to sever the lateral would be only a superficial cut if applied to the medial division. If the results of the preceding series of experiments had been due to trauma to the root as a whole the same results should be obtained when that trauma involved the medial instead of the lateral side. One such experiment was performed. In Cat 64 the right seventh lumbar dorsal root was prepared for the experiment and stimulated as in the preceding experiments. Struggling, changes in respiration and a pressor vasomotor reflex resulted. Then a cut was made along the medial side of the entering root fully equal to that made in five out of six of the experiments on the lateral division. This cut, which must have traumatized the root as a whole as much as did the lateral cuts, was nevertheless without effect on the pain reflexes. Stimulation of the damaged root brought out the struggling, changes in respiration and pressor reflex just as it did before the lesion, and these pain reflexes were undiminished—figure 2c. It is evident, therefore, that the injury to the lateral side of the root had the effect of eliminating the pain reflexes not because of general trauma to all the fibers of the root, but because of the specific lesion in the lateral division of the root which involved the unmyelinated fibers.

In two other experiments the cut on the medial side was made more boldly and extended into the cord along the line *B* in figure 1, cutting off the medial division of the root as it runs obliquely upward into the fasciculus cuneatus. In one of these, Cat 61, a study of serial sections shows that the medial division of each and every radicle of the left seventh lumbar root was completely severed along the line of *B* in figure 1. In the other, Cat 66, the medial division of the highest radicles of

the root were incompletely severed and a considerable number of medullated fibers from these radicles ran cephalad on the lateral side of the cut to reach the fasciculus cuneatus. It is true that a few medullated fibers make their way into the posterior gray column lateral to the line of the incision. But it may be conservatively estimated that in Cat 66 more than 75 per cent of the medullated fibers of the root were cut, and in Cat 61 more than 90 per cent, and yet in neither of these experiments were the pain reflexes abolished. Figure 3 represents a tracing from Cat 61 and shows that after section of the majority of its medullated fibers (estimated at 90 per cent) stimulation of the seventh lumbar root gave a good pressor reflex. It is true that the rise was 30 per cent less than that produced by the same stimulation before the medial cut was made, but this was to be expected. It is remarkable that so extensive a cut could be made so close to the lateral division of the root without traumatizing that part of the root more extensively than is indicated by a 30 per cent decrease in its conductivity. In Cat 66 the pain reflexes were not at all decreased by section of the medial division of the root. This experiment shows conclusively that after the great majority of the myelinated fibers have been cut stimulation of the root still gives a good pressor reflex. This is very significant in connection with the fact that division of the majority of the unmyelinated fibers through a relatively small lesion on the later side of the root completely abolished the pressor reflex. In Cat 66, after section of the medial division of the root stimulation still caused an increase in depth of respiration and doubled the rate. We have no record of the respirations in Cat 61.

FIG. 3. Carotid blood pressure tracing Cat 61. Strong faradic stimulation of the left seventh lumbar dorsal root after the medial division had been completely severed along the line *B* in figure 1.

From the results of these last two experiments it may be concluded that the afferent impulses which cause changes in respiration and the pressor vasomotor reflex are not conveyed by the medial division of the dorsal roots.

INTERPRETATION OF RESULTS

We have shown that the afferent impulses producing struggling, increased rate and depth of respiration and the pressor vasomoror reflex are conducted along the lateral division of the dorsal root and not along the medial division. Now, practically, all of the fibers in the medial division are myelinated and the great majority of those in the lateral division are unmyelinated. Our results may, therefore, be restated as follows: The reflexes mentioned are abolished whenever most of the unmyelinated fibers are cut, but remain unaffected when a majority of the myelinated fibers have been divided. The conclusion cannot be avoided that the afferent impulses bringing about these reflexes are nediated by unmyelinated fibers.

Struggling and the changes in respiration and blood pressure which have been described have always been regarded as reflexes produced by painful afferent impulses and we believe that we may safely conclude from our experiments that the afferent impulses underlying conscious pain also reach the cord by way of the unmyelinated fibers, although in the nature of things this can never be absolutely proven by animal experiments.

Pain belongs to Head's group of protopathic sensations. He believes that the sensations of this group—pain and the temperature sensations aroused by objects under 22° or over 40°C.—are mediated by a special set of nerve fibers. As has been seen in a preceding paragraph, the most striking parallel exists between what is known of the protopathic fibers and what we have learned concerning the unmyelinated fibers. In this paper we have presented evidence that at least the pain element of protopathic sensibility is conveyed by these fibers. On the basis of the rapidly accumulating evidence, we believe that the unmyelinated fibers mediate not only pain, but the protopathic temperature sensations as well.

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IV. DIFFERENCES IN RHYTHMICITY AND TONE IN DIFFERENT PARTS OF THE WALL OF THE STOMACH

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Before taking up the differences observed in the stomach, it may be of interest to review briefly the theoretical considerations that led up to the work. When it was seen that the rate of rhythmic contracton in the small intestine varies inversely as the distance from the pylorus (1), the next question to arise was: If this part of the primitive intestinal tube behaves in this way, how about the other parts? Might not the tract have been constructed originally so that the rate would be highest at the pharynx and lowest at the anus? Although this question cannot be answered satisfactorily as yet, there is considerable evidence in favor of such a view. For instance, the rates of contraction in different parts of the colon (of the rabbit and cat) fit quite well into a prolongation of the curve plotted from the rates of the small bowel (2). The rhythm varies (in the rabbit) from 6 to 10 per minute near the cecum to from 3 to 5 per minute near the anus. It is impossible to say much about the esophagus of mammals because, in them, that tube is made up almost entirely of striated muscle, and the smooth fibers, with which we are concerned, appear only in the lower third or fourth. Longitudinal segments from this region near the cardia (in rabbits and cats) showed a high rhythmicity when placed in aerated Ringer's solution. The fastest rate seen in the cat was 14 per minute; in the rabbit it was sometimes as high as 19 per minute. It should be noted that this is a higher rate than that ever seen in the duodenal segments (15 to 17.5 per minute).

We can compare the rhythm of different regions of the esophagus only in those lower animals in which the tube is made up entirely of smooth muscle. This is the case in the frog. Stiles found in the esophagus of this animal that the rhythmic activity is more marked and

regular than in any other part of the digestive tract; also, that the rate of contraction (in the esophagus) varies inversely as the distance from the pharyngeal end (3). I have confirmed these findings in a number of frogs; and, although my tracings are not so regular as Stiles', they show the difference in rate very clearly. I have found similar differences in longitudinal segments from the esophagus of a small grass snake (species unknown). The contractions in the intestinal segments from the frog unfortunately were so irregular that I could not establish a further gradation of rhythm from the end of the esophagus down to the cloaca. The only thing that can be said is that the esophageal rates were generally faster than those of any part of the bowel.

Even if further work on such animals should show definitely a gradation of the rhythmic activity from pharynx to anus, we would still have to explain the slow rhythm of the gastric waves in mammals: from 3 to 4 per minute in the rabbit, dog and man, and from 4 to 6 per minute in the cat. A possible way out of this difficulty was suggested to me by the literature on another muscular tube—the heart. Gaskell taught us to view that organ as an elaboration of a simple tube which had become twisted on itself, and had bulged in places. There the muscle became specialized that it might contract and empty the cavities more quickly. "The development of this nearer approach to striated muscle is made at the expense of the original rhythmical power" (4).

THE PRIMITIVE TUBE

A glance at figures 1 to 34 in Oppel's Comparative Histology (5), or at plates 18 to 33 in Huntington's book (6), will show how the stomach also has been evolved from a simple tube, first by an enlargement, secondly, by a bending of the pylorus towards the cardia, and thirdly, by the addition of cecal pouches. The stomach of the eel consists almost entirely of such a pouch, which has grown from the convex side of a bend in the original tube (see fig. 1, *D*). It is very obvious, in such a stomach, that the primitive tube is to be found along the lesser curvature. Even the complicated stomachs of ruminants can be resolved into a series of ceca arranged along the orginal tube (fig. 1, *E*). That part of the fundus to the left of the cardia in the human stomach represents such a cecum, which, very early in life, grows out from the greater curvature (7). The stomach of a 10 mm. human embryo is made up of three parts: the expanded, conical, lower end of the esophagus, the long tubular antrum, little larger than the adjacent duodenum, and a

small fundus (fig. 1. *F*). The end of the esophagus meets the antrum at the incisura angularis. Later, the fundus grows at the expense of the other two parts, so that, in the adult, the end of the esophagus is represented only by the cardiac antrum and that prolongation along the lesser curvature which forms the gastric canal; while the pyloric antrum makes up a much smaller part of the stomach than it did originally (8).

The "Primitive Tube," accordingly, must be looked for along the lesser curvature. It is suggestive that this part of the stomach is

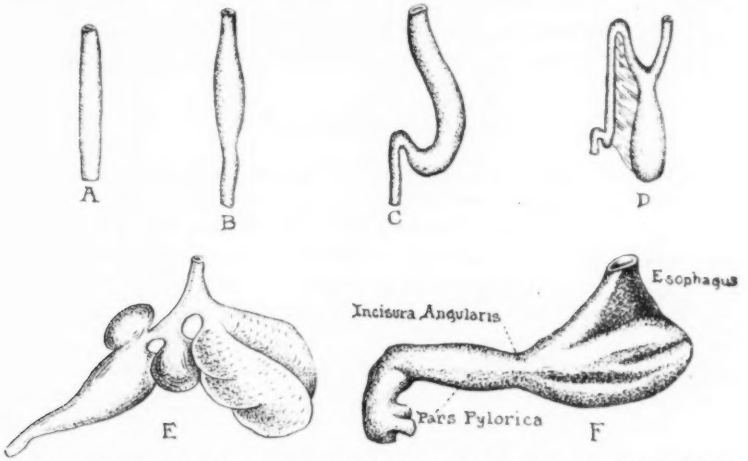

FIG. 1. To show the development of the stomach. *a*, Stomach of the pickerel (Nuhn); *b*, stomach of Proteus anguineus (Nuhn); *c*, stomach of Scincus ocellatus (Nuhn); *d*, stomach of the eel (Huntington); *e*, scheme of the ruminant compound stomach (Nuhn); *f*, stomach of a 10 mm. human embryo (Lewis).

lined by an epithelium differentiated least of all from that of the intestine. This point has been remarked upon by several men in discussing the mucous membrane of the pyloric antrum. The glands around the cardia are apparently little more than sluggish pyloric glands (9); and "it is almost the rule for the greater part of the mucous membrane along the lesser curvature to be of the pyloric type" (10). A similar arrangement is found in most of the domestic animals, that is, the lesser curvature is lined only by cardiac and pyloric glands (11).

To be sure, we must be careful in comparing conditions in two organs so different in function as are the heart and stomach. One has been

specialized to pump blood rapidly; the other serves largely as a reservoir, a hopper for the bowel, where the waves do more mixing than propelling. Yet they have both been evolved from simple tubes of rhythmic muscle, and it seems to me that the analogies are close enough to make us eager to examine the stomach from a point of view which has done so much to advance our knowledge of the physiology and pathology of the heart. In the stomach, we should expect to find the most rhythmic tissue at the cardia and along the lesser curvature. The least rhythmic tissue might be in the fundus and along the greater curvature. Such differences, if present, might go far to explain the origin and peculiarities of gastric peristalsis. I wish to show now to what a considerable extent these expectations have been fulfilled.

TECHNIC

After some experimenting, good records were obtained from longitudinal strips of muscle from different parts of the stomach of the rabbit, cat, dog, and man. With a razor, parallel cuts 3 to 5 mm. apart were made just to the mucosa. A narrow strip of muscle 2 cm. long was then lifted up, after cutting through the submucosa with a fine pair of scissors. The only place in the rabbit where this was impossible was along the canalis gastricus from the cardia to the incisura angularis. Here I could find no line of separation, so the muscular strips from this part of the lesser curvature were studied with mucous membrane attached (fig. 2, *A* and *B*). A separation could be made in the cat, dog and man, although it was more difficult in this region than in the rest of the stomach. This close attachment of the mucosa to the muscle brings to mind a similar arrangement of skin and fascia in the palm of the hand, which enables us to grasp things firmly. In the same way, in the stomach, this intimate relation between muscle and mucous membrane may be essential to the formation of the canalis gastricus, through which fluids flow along the lesser curvature (12). A localized contraction on the greater curvature might not show at all on the inside of the stomach, as the mucosa there is redundant, and but loosely attached to the muscle.

Separation of the strips was very easy in the pyloric antrum, and the laxity of the submucosa in that region was striking in all the animals studied.

The strips were usually immersed in warm aerated Ringer's solution and studied at once, but they can be kept in the icebox for four or five

days. It is remarkable that daily tracings from the same set of strips showed that they might beat even better on the second or third day than on the first. This seemed to be due to a loss of inhibition, as the strips generally began beating more promptly after immersion; the rhythm often was faster, and the records more regular. A great deal of patience was needed with fresh strips, as many of them did not show activity until they had been in the warm Ringer's solution for an hour or more. Even then, one part of barium chloride to 25,000 of the solution often had to be added before some of them would beat. The temperature of the Ringer's solution was kept between 37° and 38°C. Ordinarily no weight was added to the light heart levers used; it was not found to be necessary.

EXPERIMENTAL DATA

The following conclusions are based upon records from the stomachs of sixteen rabbits, eight cats, nine dogs and one man. This material seemed to be sufficient, as most of the data were in such entire agreement. Longitudinal strips from the two curvatures have been used almost exclusively. Some work was done with pieces cut longitudinally midway between the two curvatures and with circular strips from different regions, but it was soon discontinued, as the only ones that showed much activity were those from the neighborhood of the cardia. The type of contraction obtained in the circular strips corresponded to that of the longitudinal ones from the same region.

The first strips to begin contracting after immersion were those from the upper end of the stomach. In the cat and dog the strip from the lesser curvature next to the cardia (fig. 3, *A*) was first, often showing activity immediately after immersion in the bath. In the rabbit, the strips from the fundus (fig. 2, *D* and *E*) seemed to recover sooner from the trauma of attachment and generally became active shortly before the cardiac strips did. Strips from the greater curvature and from the antrum (particularly in the rabbit) often took an hour or two to get started, and even then some did not contract well. Thus, out of eleven strips from the rabbit's antrum, only two showed rhythmic activity.

It should be emphasized that the only strip that could be counted upon in every stomach to give regular, typical tracings was that cut from the lesser curvature near the cardia (*A*, figs. 2 and 3). *This region showed the greatest tendency to rhythmic contraction of any part of the stomach.*

THE DIFFERENT TYPES OF CURVES

The curves traced by strips from certain regions of the stomach were so characteristic that there was little need for labelling some of them. This might be said particularly about the records of the strip from the lesser curvature of the rabbit near the cardia. The individual

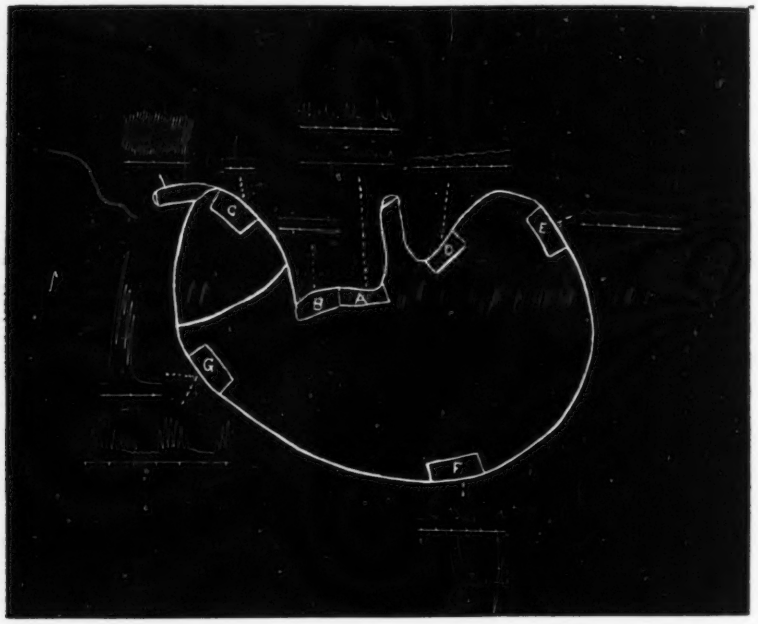

FIG. 2. Diagram of the rabbit's stomach showing the location of the principal strips studied, together with specimen tracings from the different regions. At *A* and *G* two characteristic types of tracing are shown. The time tracing represents 30 second intervals. A short strip of duodenal tracing is inserted for comparison.

contractions of this strip could generally be recognized by the almost vertical rise and the sharp peak caused by the immediate relaxation (see figs. 2 and 4). This type of curve often shaded into another very regular form, particularly when the rate became faster, or after the addition of 1:25,000 $BaCl_2$ (fig. 4). In the cat and dog, the curves from this region could be recognized not only by the sharper apices to the

contractions and the more rapid rate, but often on account of the peculiar tonus waves depicted in figure 5.

Strips from the lesser curvature (*B*, figs. 2 and 3) beat with a very small amplitude in all the animals. In the rabbit, the waves could sometimes be made out only by using a hand lens. Many strips did not beat at all. The possible reasons for this will be taken up later.

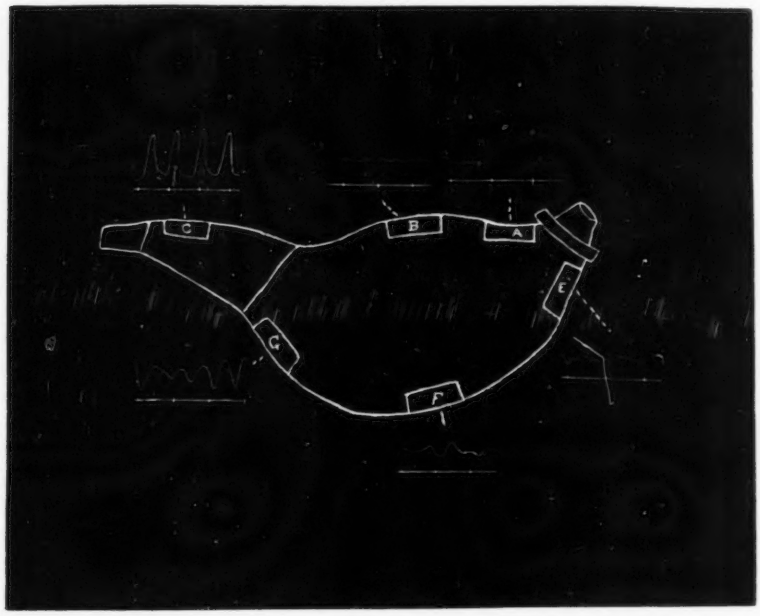

FIG. 3. A diagram of the cat's stomach to show the location of the principal strips studied and the types of tracing peculiar to the different regions. This diagram will serve also for the location of strips in the dog's stomach. The time tracing repres nts 30 second intervals.

In the rabbit, strips next to the cardia on the side towards the fundus (fig. 2, *D*) gave tracings very different from those just described for strip A on the side of the lesser curvature. They were characterized by marked irregularities of tone, rhythm and amplitude. Sections from the rest of the fundus behaved in much the same way. In the cat and dog there was less difference in the behavior of the strips on the two curvatures next to the cardia (fig. 3, *A* and *E*). This is to

be expected when we remember that their stomachs have almost no fundus and that they are simpler and less differentiated from the original tube.

Strips from the middle region of the greater curvature in all the animals (*F*, figs. 2 and 3) varied a good deal in their reactions. Some did not beat at all, others gave fair tracings, while a few were quite regular. The best curves in the rabbit were seen after the tone had been raised by barium chloride; and particularly in strips which had been on ice from 24 to 48 hours. They never resembled the typical ones from the cardia however. Ordinarily, the waves were large, rounded and uneven. They were even larger and more rounded in the cat and dog.

Strips from the greater curvature near the antrum in the rabbit's stomach gave peculiar curves characterized by regularly recurring groups of waves with a wide amplitude. Sometimes the muscle strip would shorten to less than half its original length. Two such curves are shown at *G*, figure 2. Particularly after the addition of a little barium to the solution, this type of curve often shaded into another, as regular and even as a duodenal tracing. This very regular curve with rapid rhythm was seen in some strips twenty-four hours old from the same region of the cat's stomach. Ordinarily in the cat and dog these strips reacted very much like those from the middle of the greater curvature.

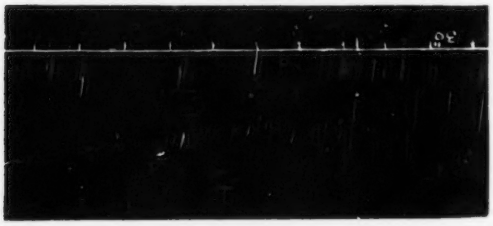

FIG. 4. Tracing from a strip from the lesser curvature near the cardia showing the change in rhythm after adding 1: 25,000 $BaCl_2$.

The type of contraction in the strips from the antrum pylori (*C*, figs. 2 and 3) was very characteristic in all the animals studied. It made no difference from what part of the antrum they were taken. The curves showed a very even base line, upon which were superimposed at regular intervals high symmetrical peaks. These are well shown in figure 6, the middle record. It was very typical even in the frog, where the great amplitude of contraction in this region was well brought out. On such tracings the antral peaks were often four times as high as the cardiac ones, in spite of the fact that the antral strip might

be only a fourth as long as the strip from the much wider cardiac end of the stomach. This difference is well illustrated by fig. 10 in an article by Woodworth (13)

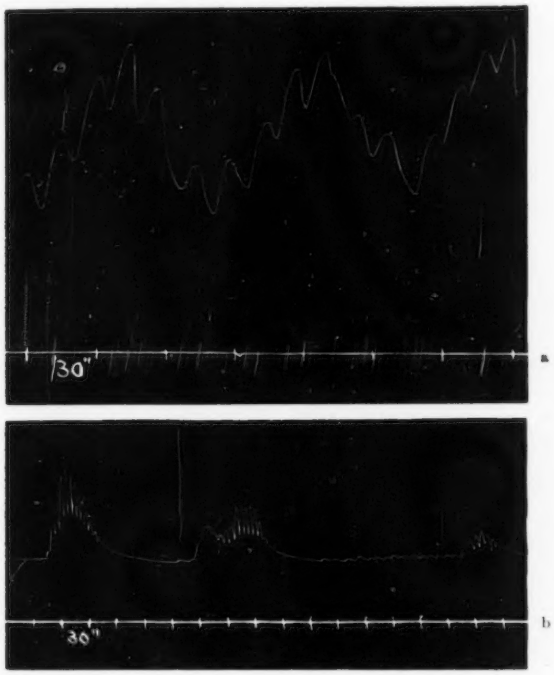

FIG. 5. *a*, Tonus waves in a record from the strip on the lesser curvature next to the cardia of a cat's stomach. *b*, From the same region in a dog's stomach.

DIFFERENCES IN THE RATE OF CONTRACTIONS

Speaking roughly, the rate varied as the distance from the cardia. The fastest rates in all the animals were observed in the tracings from strip A on the lesser curvature. In the cat, this strip contracted from 4 to 8 times per minute. In the rabbit and dog there were two or three different types of curve with different rates. Often when the strips first began to beat after immersion, the rate would be from 2 to 5 per minute. After awhile the contractions would become smaller and more

frequent, and the rate would change to from 9 to 14 per minute, usually about 11. Three strips beat 20 times per minute for short intervals. The rate in the dog was usually from 8 to 13 per minute, but in a few animals it was from 4 to 7 per minute. At the other end of the stomach, in the antrum, the rates varied from 1 to 4 per minute in all the animals. The rates of the other strips ranged between these two extremes: usually from 4 to 9 per minute. One exception must be made in regard to the strip on the greater curvature next to the antrum in the rabbit(*G*, fig. 2). Here the rate was often from 9 to 12 per minute. The possible significance of this will be discussed later.

DIFFERENCES IN TONE

Differences in tone were observed while studying the strips. When the cuts were made through the muscle on the lesser curvature, the edges pulled apart farther than they did on the greater curvature. Strip A on the lesser curvature was always much smaller than the hole from which it was removed, but strips E and F on the greater curvature might be even larger than the hole, if care were not taken to avoid all traction. Strips E and F would often lie flat when put into Ringer's solution, but strips A, B and G generally curled up tightly.

A high tone on the lesser curvature might have something to do with the poor amplitude of contraction in the strips from this region. I have commented elsewhere (14) upon the fact, so often observed with smooth muscle, that as the tone rises, the amplitude of contraction falls until rhythmic activity may cease entirely. The larger amplitude of contraction seen in the strips from the greater curvature agrees perfectly with the supposition that the tone is low in that region. The great amplitude of contraction in the strips from the antrum and preantrum is probably due to other factors, as the tone seemed to be high. The fibers of the muscle might be longer or more nearly parallel in this region. Such histological differences have been found to explain differences in the reactions of the frog's sartorius and gastrocnemius (15). It is suggestive that McGill (16) has noticed histologically a tendency to almost total contraction of muscle fibers in the pyloric ring.

Tone, unfortunately, is a vague and often misused term. Sherrington has shown recently (17), that many of the phenomena attributed to it are really what he calls "Postural" changes, that is, there is an adjustment of the contractile length of the muscles without necessarily

an alteration of tension. Such adjustments must take place constantly in the fundus of the stomach so that it can maintain a steady even pressure on the material that is being fed into the rhythmically contracting pyloric mill. This may explain the marked tendency of excised strips from the rabbit's fundus to contract down to about one-half of their original lengths after they have been in the warm Ringer's solution for from 30 to 60 minutes. After this, they seldom relaxed or showed much rhythmic activity. It is interesting to note the resemblance of the tonus waves in a strip from the neighborhood of the dog's cardia (fig. 5b) to those observed by Fano, Porter (18) and others in the auricles of the toad and terrapin. Such changes have been noticed near the cardia in the intact stomach also (19).

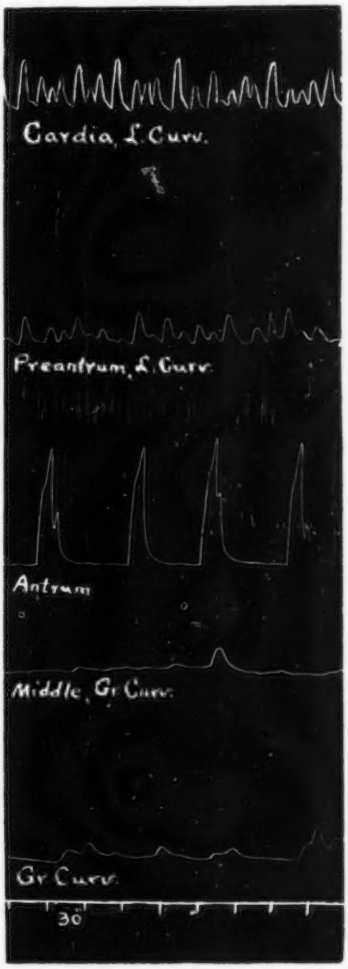

FIG. 6. Records from five strips from different parts of the dog's stomach. Shows typical contractions from the pyloric antrum.

THE HUMAN STOMACH

The kindness of Doctors Baxter and Brill enabled me to get the stomach of a man within a half hour after death from nephritis. Strips from this stomach reacted very much like those already studied. Figure 7a shows the small, regular and rapid rhythm in the cardiac strip from the lesser curvature. The rate varied between 5 and 12 per minute. The next piece on the lesser curvature showed a few contractions, only after barium was added. The strips from the preantrum on the lesser curvature contracted very much like those from the greater curvature in some

cats. The amplitude was large, the rhythm slow and irregular. The strip from the antrum pylori on the greater curvature showed the usual type of curve for that region. Strips from the greater curvature showed less rhythmicity than did those from the lesser curvature.

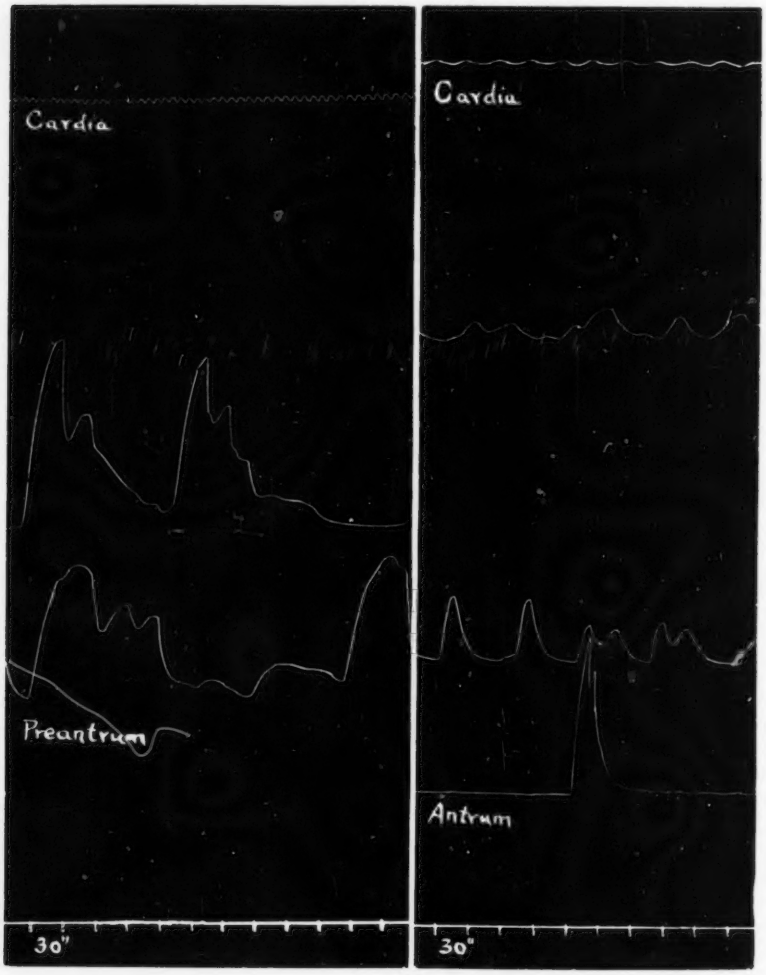

FIG. 7. *a*, Records from four strips from different parts of the lesser curvature of a human stomach. *b*, Four strips from the greater curvature.

DISCUSSION

As was expected, marked differences have been found in the behavior and in the rhythmicity of the strips. Some of these peculiarities, such as the high rhythmicity of strip A, the grading of the rhythm downwards towards the pylorus, and the differences in tone on the two curvatures are explainable on the basis of the theory that gave rise to the work. Other features, such as the low rhythmicity of strips from the middle of the lesser curvature, the promptness with which strips from the rabbit's fundus began contracting after immersion, and the high rhythmicity of the preantral strip on the greater curvature are rather against the view that the rhythmicity should vary inversely as the distance, spacially or embryologically, from the primitive tube.

There are probably other modifying factors present, such as those tending to fit the muscle in the different regions to the different types of work that have to be done. Such a factor might account for the marked differences between the behavior of strips from the pyloric antrum and from the body of the stomach. A remnant of the original tube itself might lose much of its rhythmicity if that function should interfere with the work to be done. This might be one explanation for the poor records obtained from strips from the canalis gastricus along the lesser curvature of the rabbit's stomach. The high rhythmicity of strip G on the greater curvature in the rabbit is not so easily explained. Auer noticed on the intact stomach that after reflex inhibition of the movements, they always returned first in the preantral ring, and he concluded that this was clearly the most rhythmic section (20). I believe, however, that it is exceeded in this regard by the cardia. These problems cannot be settled on the basis of differences in rhythmicity alone. More light must be obtained by studying the irritability, latent period, conductivity, etc., in the different regions. For instance, as will be seen in the next paper, a comparison of the latent periods in different parts of the stomach seems to support the original theory even more than has the study of differences in rhythmicity.

It took many experiments and years of discussion to establish the fact that the stomach can perform its functions quite satisfactorily and normally after section of all extrinsic nerves (21). The observations presented in this paper show now that local peculiarities of tone and rhythmicity may have much to do with directing and modifying the peristaltic wave as it travels over the stomach. A glance at one of

Groedel's (22) illustrations made up of the superimposed outlines of a dozen serial radiographs of the same human stomach will show how little the lesser curvature, as far as the incisura angularis, is affected by the peristaltic wave. Appearing at a variable distance from the fundus, the waves seem to travel almost entirely along the greater curvature, getting deeper as they approach the antrum. At that point, their character changes markedly; they involve the whole circumference of the stomach and are so deep that they sometimes meet in the center.

It seems to me that these local differences in the peristaltic wave correspond perfectly to the regional peculiarities of tone, rhythm and amplitude of the tissue through which it must pass. As in the heart, so here, the waves probably have their origin in the most highly rhythmic area. Similarly, again, the gradation of rhythmicity from cardia to pylorus may have much to do with maintaining the downward course of the waves.

Conduction must be very different in the two organs, as, in the stomach, the waves keep traveling quite normally after several encircling cuts have been made down to the mucosa (23). Moreover, the pyloric portion of the stomach in dogs continues to functionate normally even after complete separation from the rest of the organ (24). There is also little interference with peristalsis in the human stomach after excising the middle portion for carcinoma (25).

Rather against the view that the waves originate near the cardia is the common observation that they seem to appear now here, now there on the greater curvature. As Cannon says, "the pulsatile source of the gastric waves has no fixed seat" (26). His work showed that a wave is likely to appear at the spot where a certain balance is struck between the tone of the muscle and the internal tension. My records from the intact intestine showed clearly that a peristaltic rush which apparently had begun in the lower ileum had really come as an unnoticed ripple, all the way from the duodenum (27). I believe that the same thing may take place in the stomach, and that ripples sent out from the cardia may deepen into large waves at the place where the conditions defined by Doctor Cannon are right. Perhaps we could see these ripples if we had better means of detecting what is going on. As Groedel (28) says, anyone watching peristalsis in the human stomach would say that the lesser curvature did not participate at all, yet good serial plates always showed waves corresponding to those on the greater curvature. Dietlen (29) has shown also that with the patient lying

down, so that the fundus is filled, small but definite waves can be seen near the cardia.

Another question that arises is, why should the rates of the strips in the rabbit and dog be so much higher than that of the intact stomach. Only in the cat do they correspond at all. It is different in the intestine, where the rates of the intact bowel and of the excised segments agree quite closely. In the rabbit it may be that the slower, more powerful contractions that were seen in many of the tracings from the cardiac strip are the ones that initiate the peristaltic waves of the stomach. The faster rates may indicate a reserve, of which the cardia has the greatest amount. It does not seem likely that the normal slow rate is due to depressor effects from the vagus as peristalsis is not quickened after double vagotomy (30). More probably the longer intervals between beats are needed for adequate rest and recovery, so that the muscle can maintain a constant level of efficiency. For the same reason, the medusae pulsate normally at only about one-seventh the rate that they are capable of maintaining under certain conditions (31).

ANATOMICAL DIFFERENCES

It is hoped that this work may induce histologists to seek for regional differences in the nervous and muscular tissues of the stomach, and to study more closely the region about the cardia and the lesser curvature. Openchowski years ago claimed that the automatism of the cardia is due to groups of peculiar ganglion cells under the serosa (32). These cells were like those found in the heart. They were distinct from Auerbach's plexus; and when they were stripped off, automatic movements ceased. This, of course, might have been due to the trauma. He found similar groups of cells near the pylorus, but there were very few in the body of the stomach. Schütz also has described such ganglia grouped about the cardia and pylorus (33). Those in the pyloric portion and fundus had connective tissue capsules. Near the cardia the cells were not in the muscle layers, as they were elsewhere in the stomach, but were in the connective tissue between the layers.

Keith (34), who has recently done some very interesting work on this problem, found the myenteric plexus well developed only in the pyloric division and along the lesser curvature. There was no localized increase or development at the point where gastric movements ordinarily seem to begin. There was, however, a "distinct modification of the musculature and myenteric plexus just distal to the ring which

marks the cessation of the esophageal epithelium and the commencement of the gastric lining. At that site there was a definite development of neuro-muscular junctional tissue—just such an area as might serve as a nodal center for the stomach." In this region in the echidna he found tissue similar to that seen in the sino-auricular node of the same animal. He believes the contractions of the stomach are there initiated. Thus, reasoning along the same lines but using different methods, Doctor Keith and I have arrived, independently, at the same conclusion.

Other differences will probably be found in the muscle itself. It is well known that there are marked differences in irritability, latent period and form of the contraction-curve between the pale and red voluntary muscles in the same frog or rabbit, between the flexors and extensors, between the abductor and adductor of the crab's pincers, or between the wing and leg muscles of an insect. Histological differences have also been found corresponding to the functional ones. The proportion between the sarcoplasm and the fibrils varies markedly in different muscles; and even in the same muscle there may be fine and coarse fibers with different degrees of irritability, so that weak and strong stimuli produce different effects. After reading the articles of Ranvier (35), Rollett (36), Grützner (37), and particularly that of Paukul (38), and seeing how remarkably striated muscles vary, not only throughout the animal kingdom but in the individual body, it seems to me unreasonable to expect that smooth muscle should have fixed properties and structure. Marked differences in the physiological properties of bits of smooth muscle from different organs are well known, but I can find very little about histological differences. McGill is about the only one who seems to have observed such details. She found in some parts of the digestive tract a persistence of the embryonic condition as shown by the distinct syncytial arrangement of the muscle fibers with both end and side anastomoses (39). Unfortunately, Doctor McGill had no reason then to note just where those bodies of embryonic tissue were found.

SUMMARY

The evidence presented suggests that the gastro-intestinal tube may originally have been constructed so that the rhythmicity of any one segment varied inversely as the distance from the pharynx.

It is proposed to study the stomach from the point of view that it has been evolved from a primitive tube much as the heart has been en-

larged and specialized. Reasoning from the grounds of comparative anatomy and embryology, we should expect to find the remnants of this tube along the lesser curvature of the stomach from the cardia to the pyloric antrum.

Excised strips of muscle from the cardiac end, and particularly that one on the lesser curvature next to the cardia, show the strongest tendency to rhythmic contraction.

Different types of tracings are peculiar to the strips from different regions of the stomach. Speaking roughly, the rate of contraction varies inversely as the distance from the cardia. The tone seems to be higher on the lesser than on the greater curvature.

Strips from the human stomach behave very similarly to those obtained from the rabbit, cat and dog.

The differences observed in the strips probably determine the direction and local peculiarities of the peristaltic wave as it sweeps over the stomach.

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